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ANNUAL REPORT

JULY 1, 1975 THROUGH JUNE 30, 1976

PART II

ANNUAL REPORT

July 1, 1975 through June 30, 1976

Laboratory of Perinatal Physiology, IRP

National Institute of Neurological and Communicative Disorders and Stroke

Report of program activities

Ronald E. Myers, M.D., Ph.D., Chief

Exposure of rhesus monkey fetuses to total asphyxia injures nuclei in the brain stem while exposure to partial asphyxia causes brain swelling and damages hemispherical structures. When fetuses are exposed to sequential episodes of partial and total asphyxia, injury to basal ganglia may occur - either alone or in combination with injury to structures in the brain stem when total asphyxia predominates, or to other structures in the hemispheres when partial asphyxia predominates. Thus, exposure to a combined sequential partial plus total asphyxia produced a spectrum of CNS injury whose derivative patterns relate to the relative predominance of one or the other types of asphyxia. This spectrum of patterns of brain pathology in the experimental animal closely resembles a similar spectrum of pathology observed in perinatally injured humans.

Various anesthetic agents have been studied with respect to effects they exert on oxygenation of the fetus. Induction of moderate I.V. pentobarbital anesthesia (35 mg/Kg) leads to improved oxygenation of the asphyxiated fetus whereas permitting the pregnant monkey to come out of anesthesia on the operative table accentuates asphyxia of the fetus presumably due to increased sympathetic nervous system stimulation. On the other hand, pentobarbital given to the mother in large doses, causes pooling of blood in the large veins of the abdomen, maternal hypotension, and progressive asphyxia of the fetus. Thus, anesthesia, when used at moderate dose levels may be used to treat asphyxia of the fetus and, when used at large dose levels, may cause asphyxia of the fetus.

Studies have continued on the physiology of late decelerations of the fetal heart. Late decelerations follow uterine contractions during labor. The monitoring of the fetus with respect to late decelerations during labor significantly contributes to prevention of fetal asphyxia and cerebral birth injury or stillbirth. Fetal heart rate monitoring has found wide use in the United States during the last several years based in part on the experimental findings of our laboratory with respect to relation between late decelerations and asphyxia of the fetus. Our studies have also correlated the magnitudes of the various parameters of late decelerations and the state of oxygenation of the fetus. More recently, we have shown that the magnitudes of late decelerations relate not only to the state of oxygenation of the fetus but also is affected by physiologic state of the mother with particular regard to duration of uterine contractions and blood pressure level. Thus, both the magnitudes of late decelerations and their slope characteristics are ultimately both defined in relation to physiologic states of both mother and fetus while the latency values with respect to timing of occurrence of the heart rate changes of the fetus remain entirely determined by the state of oxygenation of the fetus. Thus, only this last parameter of the late decelerations of the fetus may be used as indicative of the state of the fetus. These relations have recently been applied in developing a computer program for use in the labor room during human delivery to provide measures of the state of oxygenation of the fetus based on the behavior

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of the heart. Such monitoring is successfully being carried out in collaboration with the Department of Obstetrics and Gynecology at the University of Alabama.

Studies on the effects of severe hypoxia on the juvenile monkey have continued. The juvenile monkey may sustain marked degrees of hypoxia (pO_2 as low as 15-20 mm Hg) without brain pathologic changes. However, when the hypoxia enters this range, slowing of the heart and decrease in blood pressure appear. In general, the more severe the hypoxia, the greater the magnitudes of these alterations in vital signs. Such vital signs changes begin when the pO_2 of the arterial approaches 22-25 mm Hg. At the same time, the electroencephalogram slows its frequency and diminishes its amplitude. Complete EEG flattening generally appears when the pO_2 has declined to 18-20 mm Hg, depending upon blood pressure level. Just as blood pressure level critically determines the behavior of the vital signs during hypoxia, so it affects the outcome with respect to individual pathologic change. If the blood pressure is artificially maintained using pressor agents, the damaging effects of severe hypoxia on the brain may be forestalled to some degree. Contrariwise, when diminished blood pressure levels which are associated with diminished cerebral perfusion are added to the effects of hypoxia, the severity of brain injury may be exacerbated.

Marked degrees of hypoxia lead to edema of the brain. This brain edema is associated with increased intracranial pressure, increased electrical impedance of gray matter tissue, and flattening of the EEG. Many animals which later go on to develop brain edema die in the early hours thereafter of respiratory arrest with signs of brain stem compression. The few animals which survive after evidences of brain swelling show brain pathology secondary to the edema itself affecting the hippocampus, areas of the occipital cortex located in relation to the edge of the tentorium, the superior surface of the cerebellum. If the severe hypoxia is combined with a brief period of anoxia as occurs when blood pressure declines to levels below which cerebral perfusion ceases the pathology may affect caudate nucleus, putamen, or globus pallidus. On the other hand, animals which have been hyperventilated and where blood pH has remained elevated, injury to white matter may ensue.

Studies on the brain pathologic effects of hypotension produced by ganglionic-blocking agents have continued. Juvenile or adult animals may survive 30-minute episodes of hypotension where the blood pressure has been maintained as low as 30-40 mm Hg. At this blood pressure level, even though the arterial blood oxygen contents are well preserved, brain edema may develop and lead to death several hours following restoration of blood pressure. The development of brain edema, again, is associated with EEG flattening, increased cortical impedance, and signs of brain stem compression and respiratory arrest. Other animals may survive such episodes of hypotension and show no brain edema or injury to the brain tissue. During the periods of hypotension themselves, increases in cortical impedance, flattening of EEG, and decreases in frequency of wave forms are seen as the cerebral blood flow decreases below critical levels. Studies have been carried out on changes in regional cerebral blood flow during arterial hypotension using both thermal dilution and autoradiographic techniques. Both techniques agree that marked depression of blood pressure leads to diminished flow of blood through the cerebral tissue. Both techniques also agree that the

decrease in flow from area to area of the brain preserves the same general patterning of blood flow as obtains during normotension, ie, there are no specific areas of the brain which show disproportionate decreases in blood flow as compared to other areas. These results speak strongly against the "border zone" hypothesis of brain injury during hypotension.

Changes in potassium concentration within the various compartments of the brain have been studied during hypoxia and anoxia. The normal potassium concentration of the cortical subarachnoid fluid as directly determined using atomic absorption spectrophotometry is 2.6 ± 0.1 mEq/l. The pressure of oxygen in the arterial blood must be reduced to 28-30 mm Hg to produce the first increase in potassium concentration in the intercellular spaces of the brain. Exposure of animals to oxygen deprivation of this magnitude also depresses cardiovascular performance and causes lowering of blood pressure. However, if the blood pressure is maintained above 80-100 mm Hg using pressor agents the K^+ concentration of cortical subarachnoid fluid remains less than 5-8 mEq/l whatever the magnitude of the depression of oxygen pressure of the arterial blood. However, if blood pressure decreases below 40-50 mm Hg, the K^+ concentration in cortical subarachnoid fluid augments to values in excess of 15 mEq/l. Cisterna magna fluid reflects changes in K^+ concentration of extracellular fluid of the brain poorly and only after long delay.

Studies with potassium selective microelectrodes have enlarged our understanding of the behavior of potassium during oxygen deprivation states. The potassium ion behaves differently according to whether oxygen deprivation is partial or total, ie, whether the animals are subjected to hypoxia or anoxia. During hypoxia, the increases in potassium ion activity in cortical intercellular fluid generally remain below 12-15 mEq/l. The severity of hypoxia required to lead to changes in potassium activity measured using ion-selective microelectrodes (20-22 mm Hg) was greater than that required to cause changes in potassium concentration in cortical subarachnoid fluid as measured using direct sampling and analysis with atomic absorption spectrometry. On the other hand, when animals were subjected to anoxia (were breathed with nitrogen or the trachea was occluded) the potassium concentration in the cortical intercellular fluid increased rapidly (within several minutes) to levels as high as 60-80 mEq/l. These changes in potassium activity were associated with EEG flattening and a rapid negative shift in DC potential. In these respects, the behavior of cortical tissue with anoxia is similar to that described with spreading depression. The characteristics of the rising and falling phases of the changes in potassium concentration during exposure to hypoxia and anoxia are currently under analysis. Preliminary results suggest that the increase in potassium concentration (its rise-time) is a complex function and cannot be described either as straight line or logarithmic. On the other hand, the decline in potassium concentration is logarithmic and the slope of the decline when plotted on semi-log scales is related to duration of the preceding period of anoxia itself - slower declines characterize longer durations of anoxia.

Juvenile rhesus monkeys overtrained on two visual discrimination tasks were subjected to 14-minute episodes of cardiac arrest induced by injections of potassium chloride. These animals remained neurologically depressed for 6-12 hours following resuscitation. Early on they failed to react to visual stimuli

at times when normal responses could be elicited to tactual or auditory stimuli. When replaced in the discrimination apparatus before about 12 hours, the animals regularly failed to respond. However, after 18-20 hours, they began to respond hesitatingly and with long intertrial intervals. The levels of performance were uniformly high even though the rates of responding were depressed. Within several days, the rates of responding also returned to normal. All animals which were resuscitated (6 out of 9) showed no long-term neurologic deficits although lesions were later demonstrated in several of the cranial nerve nuclei (including the IIIrd, IVth and VIth). These behavioral studies confirm earlier pathologic studies which demonstrated minimal pathologic changes in animals subjected to episodes of circulatory arrest lasting less than about 12-14 minutes.

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| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | | U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | | PROJECT NUMBER Z01 NS 01388-11 LPP | |
| PERIOD COVERED July 1, 1975 to June 30, 1976 | | | | | |
| TITLE OF PROJECT (80 characters or less) Perinatal Asphyxia and its CNS Consequences | | | | | |
| NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT PI: R. E. Myers Chief, Lab. of Perinatal Physiol. LPP NINCDS | | | | | |
| COOPERATING UNITS (if any) Mt. Sinai School of Medicine, New York University of Puerto Rico, San Juan | | | | | |
| LAB/BRANCH Laboratory of Perinatal Physiology | | | | | |
| SECTION | | | | | |
| INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20014 | | | | | |
| TOTAL MANYEARS: 1.10 | | PROFESSIONAL: .65 | | OTHER: .45 | |
| SUMMARY OF WORK (200 words or less - underline keywords) This project investigates the <u>clinical</u> and <u>neuropathologic alterations</u> produced in the fetus by <u>asphyxia</u> during different stages of pregnancy, at the time of birth, and during the newborn period. It attempts to correlate the patterns of brain injury produced with the physiologic changes occurring in the fetus during asphyxia. | | | | | |

Project Description:

Objectives: To determine the clinical and neuropathologic changes produced by asphyxia of the fetus or newborn and to correlate the patterns of neuropathologic change with physiologic alterations observed at the time of injury.

Methods Employed: Monkey fetuses are delivered by Cesarean section and subjected to episodes of total asphyxia (anoxia) for predetermined lengths of time by placing a rubber sac over the fetal head and clamping the umbilical cord. In some instances, an effort is made to produce the maximum injury compatible with survival. Other fetuses are subjected to episodes of partial asphyxia (hypoxia) produced in a variety of ways most of which are characterized by impaired intervillous space perfusion. The cardiovascular, acid-base, respiratory gas, and respiratory changes which occur during and after the asphyxia are recorded and later correlated with the clinical and brain pathologic abnormalities produced.

Major Findings: With the varying durations and magnitudes of asphyxia, varying severities of brain damage are produced. The most common clinical alteration observed following anoxia is widespread sensory loss manifested by abnormal placing reactions, altered sucking and swallowing, altered vocalization, and ataxia and motor weakness. With more marked damage, the animals show changes in postural tone and in use of the extremities. Total paralysis may be seen and the extremities may show marked extensor hypertonus. Pathologically, destructive changes affect various brain stem and thalamic nuclei. Damage to cortex or basal ganglia rarely occurs with anoxia.

Episodes of partial asphyxia (hypoxia) do not damage lower brain stem structures, but, instead, affect various hemispherical structures including cortex, hemispherical white matter, and basal ganglia. These hemispherical patterns of injury resemble the patterns of injury observed after human perinatal asphyxia.

Significance: The intent is to reproduce the neuropathology of human cerebral palsy and mental retardation. Ulegyria, atrophic cortical sclerosis, lobar atrophy, status marmoratus, periventricular leukomalacia, porencephaly, hydranencephaly, and microcephaly have all been experimentally reproduced.

Proposed Course of Project: We will attempt to determine the critical parameters which lead to deterioration of vital functions of the fetus during asphyxia and to relate these physiologic alterations to the patterns of brain injury produced. Efforts are under way to develop techniques to prevent the deterioration of the fetus when asphyxiated and to prevent or postpone the occurrence of brain injury.

Publications:

Myers, R.E.: Perinatal asphyxia: The Neurologist's viewpoint. In Adamsons, K. and Fox, H.A. (Eds.): Preventability of Perinatal Injury. New York, Alan R. Liss, Inc., 1975, pp. 59-93.

Myers, R.E.: Perinatal asphyxia: Immediate and long-term effects. In Kondo, S., Kawai, M., Ehara, E. and Kawamura, S. (Eds.): Proceedings From The Symposia of The Fifth Congress of The International Primatological Society, Nagoya, (1974), Tokyo, Japan Science Press, 1975, pp. 369-384.

Myers, R.E.: Response of the primate fetus to asphyxia. In Hafez, E.S.E. (Ed.), The Mammalian Fetus: Comparative Biology and Methodology. Springfield, Charles C. Thomas, Publ., 1975, pp. 320-345.

Myers, R.E.: Production of fetal asphyxia by maternal psychologic stress. In Rippmann, E.T., Stamm, H., McEwan, H.P. and Howie, P. (Eds.): Progress in EPH-Gestosis. IIIrd International Symposium on EPH-Gestosis, Glasgow, (1973), Basel, Organisation Gestosis Press, 1975, pp. 3-15.

Myers, R.E.: Fetal asphyxia due to umbilical cord compression: Metabolic and brain pathologic consequences. Biol. Neonate. 26: 21-43, 1975.

Ginsberg, M.D. and Myers, R.E.: Clinical and neuropathologic aspects of fetal brain injury following maternal carbon monoxide intoxication. Neurol. 26: 15-23, 1976.

Cosmi, E.V., Condorelli, S., Tonelli, F. and Myers, R.E.: Reanimacion del recién nacido de oveja tras asfixia aguda endouterina. Clin. e Invest. en Gynecol. y Obstet. 2: 59-68, 1975.

Adamsons, K. and Myers, R.E.: Circulation in the intervillous space: Obstetrical considerations in fetal deprivation. In Grunwald, P. (Ed.): The Placenta and Its Maternal Supply Line: Effects of Insufficiency on the Fetus. Lancaster, England, Medical and Technical Publ. Co., 1975, pp. 158-177.

Joelsson, I., Myers, R.E. and Adamsons, K.: Adrenergic agonists and fetal oxygenation in the primate. In Kondo, S., Kawai, M., Ehara, E. and Kawamura, S. (Eds.): Proceedings From The Symposia of The Fifth Congress of The International Primatological Society, Nagoya, (1974), Tokyo, Japan Science Press, 1975, pp. 395-407.

Comas-Urrutia, A., Adamsons, K. and Myers, R.E.: Response of the primate fetus to intraamniotic saline injection. Am. J. Obstet. Gynec. 122: 549-554, 1975.

Mueller-Heubach, E., Adamsons, K. and Myers, R.E.: Production of disseminated intravascular coagulation in monkeys by injection of cell-free extracts of placenta. Mount Sinai J. Med. 42: 415-423, 1975.

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| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do not use this space) | U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 NS 01464-10 LPP |
| PERIOD COVERED July 1, 1975 to June 30, 1976 | | |
| TITLE OF PROJECT (80 characters or less) Biochemistry of Brain Recovery from Hypoxia and Anoxia | | |
| NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT PI: M. Yamaguchi Visiting Associate LPP NINCDS OTHER: R. E. Myers Chief, Lab. of Perinatal Physiol. LPP NINCDS | | |
| COOPERATING UNITS (if any) None | | |
| LAB/BRANCH Laboratory of Perinatal Physiology | | |
| SECTION | | |
| INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20014 | | |
| TOTAL MANYEARS: 2.5 | PROFESSIONAL: 1.5 | OTHER: 1.0 |
| SUMMARY OF WORK (200 words or less - underline keywords) This work investigates alterations in activity or concentration of various <u>enzymes</u> and <u>substrates</u> of the brain in response to episodes of <u>anoxia</u> or <u>hypoxia</u> . Changes in content of water and <u>electrolytes</u> also are examined in cortex, basal ganglia, and hemispherical white matter. Tissues are examined during the course of the episodes of anoxia and hypoxia and also following resuscitation and recovery. A particular attention is paid to comparisons of changes in enzyme activities and substrate concentrations in brains of animals which develop <u>brain edema</u> and those which show normal recovery. | | |

Project Description:

Objectives: To define and compare the biochemical changes produced in the brain by hypoxia and anoxia and to relate these changes to development of brain edema.

Methods Employed: Monkeys under barbiturate anesthesia are subjected to episodes of anoxia or hypoxia. The blood pO_2 , pCO_2 and pH are analyzed as are blood pressure, heart rate, respiration and EEG. Tissue samples are taken from the brain after different periods of treatment and frozen in liquid nitrogen. The frozen tissues are analyzed for ATP, lactate, pyruvate, and cyclic AMP. Fresh but not frozen tissues are analyzed (ATPase, Alkaline and Acid Phosphatases, etc.). Tissue water content is also determined. Blood glucose, lactate and pyruvate are analyzed enzymatically.

Major Findings: Differences between anoxia and hypoxia: Anoxic brain tissue shows marked decreases of ATP and moderate increases of lactate. Hypoxic brain tissue shows much greater accumulations of lactate, increased alkaline phosphatase activity, but only slight decreases of ATP content.

Brain swelling after hypoxia: Brain tissue samples 5 hours after exposure to moderate hypoxia show near normal ATP and lactate contents and normal enzymatic activities. Brain tissue samples 5 hours after exposure to more marked hypoxia show increased water content of cortex, marked accumulation of lactate, decrease in ATP, and increased alkaline phosphatase activity. These changes are associated with developing brain edema.

Effects of glucose administration on response to anoxia: Administration of glucose prior to exposure to anoxia greatly diminishes tolerance of brain to injury and alters resulting brain pathology. Biochemical studies with food-deprived and glucose-infused animals demonstrate much greater increases of lactate content in brain tissue of animals subjected to arrest of circulation following glucose infusion compared to that in food-deprived animals. A close correlation was observed between magnitude of lactate accumulation in brain and later development of brain edema and tissue necrosis. Studies with monkeys and rats demonstrate a correlation between serum glucose concentration prior to arrest and magnitude of accumulation of lactate in cerebral tissue following 10 minutes of circulatory arrest.

Significance: Significant changes were observed in content of ATP and lactate in relation to the several oxygen-deprivation states. Anoxia leads to major depletion of ATP and only moderate lactate accumulation whereas hypoxia causes less marked decreases in ATP but marked accumulation of cerebral tissue lactate. Post-hypoxic swelling of the brain is closely correlated with accumulation of lactate in cerebral tissues at concentrations greater than 15 meq/kg. Infusion of glucose prior to exposure to oxygen deprivation leads to excessive lactate accumulation in cerebral tissues and diminished tolerance to oxygen deprivation and enhanced brain pathologic changes.

Proposed Course of Project: More complete studies are planned to define the temporal and quantitative relations between accumulation of glycogen, glucose, and lactate in serum and cerebral tissues in these various deprivation states. Studies correlating lactate accumulation in brain tissue and magnitude of brain edema and pathology will be carried out.

Publications:

Rivera, A., Martinez de Jesus, J., and Myers, R.E.: Changes in tissue glycogen of recovering asphyxiated newborn monkeys. Biol. Neonate. 27:279-288, 1975.

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| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do not use this space) | U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 NS 02020-04 LPP |
| PERIOD COVERED July 1, 1975 to June 30, 1976 | | |
| TITLE OF PROJECT (80 characters or less) Pathogenesis of Brain Injury Produced by Cardiovascular and Pulmonary Diseases | | |
| NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT PI: R. E. Myers Chief, Lab. of Perinatal Physiol. LPP NINCDS OTHER: W. F. Blank Staff Associate LPP NINCDS | | |
| COOPERATING UNITS (if any) None | | |
| LAB/BRANCH Laboratory of Perinatal Physiology SECTION | | |
| INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20014 | | |
| TOTAL MANYEARS: 2.20 | PROFESSIONAL: 1.28 | OTHER: .92 |
| SUMMARY OF WORK (200 words or less - underline keywords) <p>This project investigates the effects of a variety of types of insults including <u>circulatory arrest</u>, <u>arterial hypotension</u>, <u>hypoxia</u>, <u>asphyxia</u>, <u>cyanide intoxication</u>, and <u>exposure to carbon monoxide</u> on the <u>juvenile rhesus monkey</u>. Alterations in cardiovascular performance and nervous system activity are examined during the insult and the distribution of patterns of brain injury produced are later determined by post-mortem examination of brain specimens. Animals are resuscitated and maintained for several weeks to determine the long-term alterations in brain morphology. Patterns of pathology produced are carefully delineated and their relation to patterns of injury produced in the human are defined. The major effort is to define the pathophysiology and pathogenesis of specific patterns of brain pathology using animal experimental models.</p> | | |

Project Description:

Objectives: To develop animal experimental models of human brain disease produced by cardiovascular and pulmonary disorders. These include studies of interferences with oxygen supply to tissues due to cessation of circulation (cardiac arrest), hypotension, pulmonary disease, myocardial abnormalities, or intoxications with agents such as carbon monoxide or cyanide.

Methods Employed: Juvenile or adult monkeys are anesthetized with pento-barbital and a femoral artery and vein is catheterized to record blood pressure and heart rate, and to withdraw blood samples for analysis of acid-base and respiratory gas values. EKG and EEG leads are placed and a variety of other physiologic parameters are monitored including cortical impedance, intracranial pressure, electrolyte composition of blood and CSF, glucose content of blood and CSF, etc. The animals are then subjected to one of the insults described above and the physiologic and biochemical changes produced are measured. Afterwards, the animals are resuscitated and any neurologic or pathologic abnormalities are evaluated and recorded.

Major Findings: The brain pathology produced by circulatory arrest depends on prior nutritional state. Animals food-deprived for 24 hours are markedly tolerant to circulatory arrest damage appearing in the brain only after 14 minutes of cardiac arrest. When damage occurs, it affects nuclear structures in the brain stem leaving hemispherical structures intact. Normally-fed or glucose-infused animals develop brain edema, altered BBB function, and widespread cerebral necrosis following short-lived (< 6 mins) episodes of cardiac arrest. Thus, serum glucose level critically determines brain pathologic response to anoxia.

The monkey can tolerate mean blood pressure lowering to 30 mm Hg over a long time without brain injury. Animals subjected to pressures lower than this die during the exposure due to myocardial failure; die in the early hours following blood pressure restoration of cardiogenic shock; or die during the first 6-48 hours due to severe brain swelling and brain stem compression. Only a small proportion of animals survive to show static, long-term lesions.

Food-deprived monkeys can sustain 14 minutes of cardiac arrest at normal body temperatures without evidence of brain injury. With longer arrest, nuclei in the brain stem show injury but a poor correlation exists between duration of stasis of blood flow and extent of injury to the brain. Normally-fed and glucose-infused animals show markedly diminished tolerances to arrest of circulation and develop brain edema and damage to structures in the hemispheres.

Hypoglycemia is associated with minor alterations in respiratory gas and acid-base state but leads to significant hypotension and circulatory impairment. When hypoglycemia animals are mechanically respired, the patterns of pathology observed affect basal ganglia, cortex and hippocampus. Significant long-term changes include convolitional atrophy and dementia. Hypoglycemia does not, by itself, lead to brain swelling.

Significance: These studies elucidating the pathophysiology and pathogenesis of patterns of brain injury caused by cardiovascular or pulmonary diseases are vitally important to development of techniques for prevention or amelioration.

Proposed Course of Project: Studies will be continued to attempt further clarification of physiologic bases for occurrence of the various patterns of brain pathology caused by respiratory and circulatory disorders.

Publications:

Kirshner, H.S., Blank, W.F. and Myers, R.E.: Brain extracellular potassium activity during hypoxia in the cat. Neurol. 25: 1001-1005, 1975.

Kirshner, H.S., Blank, W.F. and Myers, R.E.: Changes in cortical sub-arachnoid fluid potassium concentrations during hypoxia. Arch. Neurol. 33: 84-90, 1976.

Gamache, F.W. and Dold, G.M.: Alterations in cerebral blood flow produced by hypotension: A comparison of methods. J. Neurol., Neurosurg., & Psychiat. 38: 765-770, 1975.

Gamache, F.W., Dold, G.M. and Myers, R.E.: Changes in cortical impedance and EEG activity induced by profound hypotension. Am. J. Physiol. 228: 1914-1920, 1975.

Gamache, F.W., Myers, R.E. and Monell, E.: Changes in local cerebral blood flow following profound systemic hypotension. J. Neurosurg. 44: 215-225, 1976.

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| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | | U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | | PROJECT NUMBER Z01 NS 02021-04 LPP | |
| PERIOD COVERED July 1, 1975 to June 30, 1976 | | | | | |
| TITLE OF PROJECT (80 characters or less) Short- and Long-term Behavioral Deficits Produced by Oxygen-deficiency States | | | | | |
| NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT PI: R. E. Myers Chief, Lab. of Perinatal Physiology LPP, NINCDS OTHER: S. Yamaguchi Research Psychologist LPP, NINCDS | | | | | |
| COOPERATING UNITS (if any) None | | | | | |
| LAB/BRANCH Laboratory of Perinatal Physiology | | | | | |
| SECTION | | | | | |
| INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20014 | | | | | |
| TOTAL MANYEARS: 1.65 | | PROFESSIONAL: .48 | | OTHER: 1.17 | |
| SUMMARY OF WORK (200 words or less - underline keywords) The project studies <u>short- and long-term effects on perceptual learning</u> <u>and memory function of CNS insults caused by a variety of cardiovascular</u> <u>and pulmonary diseases and/or drug intoxications.</u> Monkeys are trained on visual discrimination tasks before exposure to injury. Afterwards, changes in performances of learned responses serve as indexes of induced psychopathology. Observations of behavior outside the formal training situation are also made. | | | | | |

Project Description:

Objectives: To determine short- and long-term effects on perceptual learning and memory function of brain insults produced by cardiovascular or pulmonary diseases and/or states of intoxication.

Methods Employed: Juvenile rhesus monkeys are trained on visual pattern discrimination tasks to choose one and to avoid the other of two visual stimuli. Food pellet rewards are used to reinforce correct responding. The monkeys are then exposed to episodes of oxygen deprivation (circulatory arrest, marked hypotension, hypoxia, total asphyxia, carbon monoxide intoxication, etc.). The animals are resuscitated and their performances again tested. If deficits occur, the time course of restoration of performance is determined. Permanent deficits are identified and quantitated.

Major Findings: Food-deprived monkeys exposed to 14 minutes of circulatory arrest and resuscitated show no deficits of visual perception or memory.

Significance: The neurologic assessment of the rhesus monkey is difficult and provides only crude measures of neurologic competency. Utilization of sophisticated behavioral test methods permits definition of subtle changes in nervous system function, both with respect to reversible impairments and also permanent deficits related to focal neurologic lesions.

Proposed Course of Project: To extend these test methods to assessment of consequences of other pathologic states.

Publications: None

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| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 NS 02022-04 LPP | | | | | | |
| PERIOD COVERED July 1, 1975 to June 30, 1976 | | | | | | | | |
| TITLE OF PROJECT (80 characters or less) Neural Mechanisms Underlying Social Behavior and Emotion in Rhesus Monkey | | | | | | | | |
| NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT <table style="width: 100%; border: none;"> <tr> <td style="width: 33%;">PI: S. Yamaguchi</td> <td style="width: 33%;">Research Psychologist</td> <td style="width: 33%;">LPP NINCDS</td> </tr> <tr> <td>OTHER: R. E. Myers</td> <td>Chief, Lab. of Perinatal Physiol.</td> <td>LPP NINCDS</td> </tr> </table> | | | PI: S. Yamaguchi | Research Psychologist | LPP NINCDS | OTHER: R. E. Myers | Chief, Lab. of Perinatal Physiol. | LPP NINCDS |
| PI: S. Yamaguchi | Research Psychologist | LPP NINCDS | | | | | | |
| OTHER: R. E. Myers | Chief, Lab. of Perinatal Physiol. | LPP NINCDS | | | | | | |
| COOPERATING UNITS (if any) None | | | | | | | | |
| LAB/BRANCH Laboratory of Perinatal Physiology | | | | | | | | |
| SECTION | | | | | | | | |
| INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20014 | | | | | | | | |
| TOTAL MANYEARS: <div style="text-align: center;">1.40</div> | PROFESSIONAL: <div style="text-align: center;">.48</div> | OTHER: <div style="text-align: center;">.92</div> | | | | | | |
| SUMMARY OF WORK (200 words or less - underline keywords) Effects of experimental brain lesions on selected <u>social and emotional</u> responses were studied in rhesus monkeys. Lesions in the <u>orbitofrontal</u> and <u>anterior temporal neocortex</u> are followed by major changes in responses, while lesions elsewhere in cortex produce only minor effects. Effects of destroying deep-lying structures such as amygdala and brain stem tegmentum are also studied. | | | | | | | | |

Project Description:

Objectives: To identify the neural mechanisms underlying social behavior and emotion in rhesus monkey.

Methods Employed: A variety of techniques are used to study social behavior and emotion in sub-human primates. In some instances, monkeys are placed in observation cages and their spontaneous patterns of vocalization are quantitated. In other instances, the general cage behavior of the animals and their reactions to challenge by the experimenter are defined. In still other instances, the animals are placed in gang cages as small social groups and their social interactions observed and quantitated.

Major Findings: Removals of cortical areas homologous to "speech" areas of human in monkeys fail to alter vocalizations. On the other hand, lesions of areas of cortex having to do with regulation and control of social behavior, including orbitofrontal and anterior temporal cortex, produce major decreases or totally abolish vocal responses. Lesions of subcortical structures reveal that amygdala and tegmentum of midbrain, both centers important to the regulation of social behavior, also alter vocal responses. Gang cage studies demonstrate major deficits in all aspects of social behavior following lesions of orbitofrontal and anterior temporal cortex. Lesions of cingulate cortex, on the other hand, produce minimal changes. Other studies with split-brain animals (destroyed crossing retinal fibers at optic chiasma) show that unilateral orbitofrontal and/or anterior temporal cortex lesions produce deficits as tested through either eye but the deficit is much greater in the half-field ipsilateral to the hemisphere of lesion. When corresponding lesions are added to the second hemisphere, additional bilateral defects are demonstrable. Thus, lesions of one hemisphere produce bilateral deficits in social behavior.

Significance: These studies which identify brain structures involved in regulating and controlling social behavior are of major importance to our efforts to understand the biologic foundations of disordered behavior.

Proposed Course of Project: Examination of other brain stem structures for their contribution to the expression of social behavior and affect.

Publications:

Myers, R.E.: Neurology of social behavior and affect in primates: A study of prefrontal and anterior temporal cortex. In Zulch, K.J., Creutzfeldt, O. and Galbraith, G.C. (Eds.): Cerebral Localization. Heidelberg, Springer-Verlag, 1975, pp. 161-170.

Myers, R.E. and Ebner, F.F.: Localization of function in corpus callosum: Tactual information transmission. Brain Res. 103: 455-462, 1976.

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| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 NS 02081-03 LPP |
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PERIOD COVERED
July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)
Cerebral Malformations Produced by Teratogenic Agents or Oxygen Deprivation

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER
PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT
PI: R. E. Myers Chief, Lab. of Perinatal Physiol. LPP NINCDS

COOPERATING UNITS (if any)
None

LAB/BRANCH
Laboratory of Perinatal Physiology
SECTION

INSTITUTE AND LOCATION
NINCDS, NIH, Bethesda, Maryland 20014

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|------------------------|----------------------|---------------|
| TOTAL MANYEARS: .94 | PROFESSIONAL: .14 | OTHER: .80 |
|------------------------|----------------------|---------------|

SUMMARY OF WORK (200 words or less - underline keywords)

Pregnant rhesus monkeys are injected with teratogenic agents or subjected to episodes of severe asphyxia in an effort to induce malformations of the brain of the developing fetus. The induced patterns of malformation are compared and contrasted with patterns of malformation which affect the human brain.

Project Description:

Objectives: To produce cerebral malformations in monkey and sheep fetus by treating the mother with teratogenic agents or exposing her to severe asphyxia at different stages of pregnancy. To study morbid anatomy of induced cerebral malformations and to relate them to the time of pregnancy during which the insulting agent was administered. The induced morphologic abnormalities will be compared with cerebral malformation of man.

Methods Employed: Pregnant rhesus monkeys and sheep are injected with MAM-acetate (methylazoxymethonal acetate, 10 mg/kg). Alternatively, the mothers are subjected to episodes of severe asphyxia. Later, the fetuses are delivered near term and the body and organ weights of the newborn are determined and the brain examined grossly and microscopically. Malformations induced are related to time of gestation during which the insulting agent acted and the malformations are classified according to time of insult and compared and contrasted with malformations in the human.

Major Findings: The toxic dose of MAM-acetate was defined for rhesus monkey and the first monkeys injected. Similarly, the magnitude of asphyxia required to produce brain injury of the fetus has been defined and fetuses are subjected to severe asphyxia during earlier gestational ages. Unexpectedly, single doses of MAM-acetate at markedly toxic levels failed to produce cerebral malformations in the monkey fetuses tested thus far.

Significance: The definition of timing and the identification of the inciting agents which lead to cerebral malformations in the primate is poorly defined. The present study should provide insights into pathogenesis and morphogenesis of cerebral malformations as they affect the primate.

Proposed Course of Project: Injection of further animals, harvesting of fetuses, weighing of organs, and examination of brains. Correlation with human findings.

Publications: None

ANNUAL REPORT

July 1, 1975 through June 30, 1976

Laboratory of Molecular Biology
National Institute of Neurological and Communicative
Disorders and Stroke

Ernst Freese, Chief

The Laboratory has investigated cellular mechanisms controlling differentiation, cellular effects of lipophilic acids and their reversibility, and viral autointerference and persistence. All investigations employed micro-organisms or mammalian cell cultures assuring the availability of large numbers of identical and often synchronized cells and allowing the biochemical investigation of cell or virus components at different times of development.

1) The investigation of differentiation has so far been limited to bacilli in which sporulation occurs almost synchronously and for which many auxotrophic and cacogenic mutants are available that have been analyzed biochemically and genetically. It was demonstrated that differentiation is not triggered irreversibly by the change of a single macromolecular species, but it comes about by a series of cellular changes each of which renders the reversion to vegetative growth more difficult. In bacilli, the complete engulfment of the prespore cell compartment by a double membrane is responsible for the ultimate commitment to differentiation. Within this new cell compartment, into which compounds can no longer be actively transported, the synthesis of new protein molecules is possible, as has been shown for a spore-specific glucose dehydrogenase which is drastically different from the glucose dehydrogenase of vegetative cells. Manganese ions are required for sporulation because they are needed as co-factor of phosphoglycerate phosphomutase, an enzyme of the glycolytic pathway.

2) Lipophilic acids, which include preservatives, antiseptics, and compounds known to cause brain damage or teratogenic effects, inhibit the growth of both bacterial and mammalian cell cultures with about the same potency. An exception is butyrate which has a higher potency for mammalian cells and causes reversible shape changes and the induction of sialyltransferase in HeLa cells. The reversion of this phenomenon has a number of interesting features which were investigated. Hexachlorophene which has strong effects on bacteria and mammalian cells, was shown to inhibit bacteria by destroying the proton gradient across the membrane and thus starving them for substrate. The compound binds so tenaciously to cells that it can not be washed off by buffers but only by highly lipophilic polymers such as bovine serum albumin. This result suggested a treatment of persons exposed to highly lipophilic toxic compounds such as kepone, PCB's, etc. 3) Defective RNA virus particles can interfere with and terminate a virus infection. During this autointerference the RNA genome of the defective particle competes with that of the normal virus for the viral replicase. Since the normal RNA genome seems to be the only template for mRNA synthesis, viral proteins become limiting; thus not only the proportion of normal viruses in the progeny is reduced but also less viral particles are released from the autointerfered cell. The affinity of RNA to the replicase apparently depends on the 3' or 5' terminus; both seem to be the same for the normal virus and all defective particles. The persistence for many years of

viruses in infected cells is a perplexing problem. At least for vesicular stomatitis virus, it is not due to the presence of viral DNA integrated into the chromosome. A method has been developed to isolate cDNA, complimentary to viral RNA, which will allow identification and innumeration of the number virus RNA particles in diseases with presumptive viral etiology.

1. Mechanisms controlling differentiation. A typical property of differentiation is the "commitment" of cells to continue their developmental processes even when they are exposed to a new environment in which they would originally have continued to duplicate (i.e. to grow vegetatively). The seemingly sudden development of this commitment with respect to a given environment has created the impression that a single cellular process may irreversibly trigger differentiation so that it would have to continue irrespective of the environment to which it would be exposed subsequently. This notion of a single irreversible trigger has been disproved by the fact that the developmental stage at which commitment occurs depends on the media from and to which cells are transferred. For example, in *B. megaterium* commitment occurs earliest with respect to addition of or dilution into a medium containing the aspartate or glutamate as sole carbon source, later with respect to glucose or sucrose, and latest with respect to a rich medium. Similar measurements in *B. subtilis* were made possible by the finding that methylantranilate (1 mM) inhibits germination of spores without interfering with growth or sporulation. Without this compound newly formed spores would immediately germinate but in its presence the frequency of refractile, octanol-resistant, and heat-resistant particles coincides and measures the fraction of committed cells. Electron microscope studies have furthermore revealed that the earliest commitment occurs before any asymmetric prespore septum has been formed indicating that some other biochemical change in the cell must be responsible for the resistance to the resumption of growth. One can conclude that cells become increasingly resistant to various external factors that could interrupt the continuation of differentiation, because cells undergo different cellular changes each of which reduces the growth response to certain compounds. However, the latest commitment, observed after cell transfer from and into any rich medium, always coincided with the closure of the forespore double membrane surrounding the forespore cell compartment. This commitment apparently results from the inability of the forespore double membrane to transport compounds actively into the forespore cell compartment, thus isolating this compartment very effectively against external influences.

As a result of the enclosure of the forespore cell compartment by a double membrane, completely new metabolic conditions are created which might lead to the expression of genetic information unexpressed in the vegetative or mother cell. Indeed we have found that a new glucose dehydrogenase is produced in the forespores. In vegetative cells, a glucose dehydrogenase appears when catabolite repression ceases. Through the use of different auxotrophic mutants, limiting the metabolism of compounds added to the medium, it could be demonstrated that one of three compounds in the Embden Meyerhof Pathway must be responsible for the repression of this enzyme. But as soon as the forespore engulfment is finished, another spore-specific glucose dehydrogenase is formed which is distinctly different from the vegetative enzyme with respect to pH stability, substrate specificity,

and electrophoretic mobility.

The specific requirement of manganese ions for sporulation has been correlated with the specific manganese requirement of phosphoglycerate phosphomutase, an enzyme of the Embden Meyerhof Pathway. The manganese requirement is a novel finding because in all other organisms so far studied 2,3-diphosphoglycerate was found to be required as co-factor; this compound had no effect on the *B. subtilis* enzyme. In the absence of manganese, cells accumulate large amounts (100 mM) of 3-phosphoglyceric acid and consequently can not sporulate. When manganese is added to such an arrested culture, 3-phosphoglyceric acid disappears, cells grow to a higher titer and subsequently they sporulate normally.

2. Effects of lipophilic acids on bacteria and mammalian cells.

Lipophilic acids inhibit the growth of bacteria and mammalian cells by a general effect whose potency increases with the lipophilicity of the compound. Since potency of this effect is the same in *B. subtilis* (but not in *E. coli*) as in mammalian cells such as HeLa, *B. subtilis* can be used as a rapid tool for the measurement of inhibitory potency. In order to compare a compound with intermediate lipophilicity and one with very high lipophilicity, deconoate and hexachlorophene were examined. Hexachlorophene was employed also because it has been reported to cause brain damage in children. This compound is so lipophilic that more than 80% of it immediately attaches to the cell membrane and remains attached upon washing of the cells. The cells behave as if they were dead. However, hexachlorophene can be removed and the cells can resume growth when they are exposed to a polymer with highly lipophilic groups, such as bovine serum albumin (1%). The findings studies have shown that about 5×10^7 hexachlorophene molecules per bacterium completely inhibit the cell duplication. Deconoate binds to cells only to a very small extent (less than 1% of the added compound) and consequently much more of this compound per ml of cell culture is needed to inhibit growth. Even the number of molecules found in the cells under conditions needed to inhibit growth is very high (2.5×10^7); presumably most deconoate molecules are not bound to the membrane (as for hexachlorophene) but are located in the cell cytoplasm. Deconoate can be removed and growth restored by a single washing with growth medium. In spite of the difference of binding, both compounds inhibit growth apparently by destroying the proton gradient across the cell membrane. They inhibit the transport of amino acids whereas they do not affect the oxygen consumption of isolated membranes indicating the continued function of the electron transport system. The results suggests that people, inadvertently exposed to a highly lipophilic toxic compound that can not be excreted in the urine, could be treated by repeated replacement of their serum albumin or by the washing of their blood with activated charcoal. The fresh blood will again establish an equilibrium with other cells and organs and thus reduce their content of toxin.

Apart from the general effect on cells, some lipophilic acids have a more specific effect already active at lower concentrations of the compound. Such an effect has been demonstrated for salicylates, which interfere with prostaglandin synthesis and for small molecular weight fatty acids, in particular butyrate, for which the mode of action is still unknown; these compounds inhibit HeLa cells much more potently than would have been expected

from their lipophilicity (partition coefficient) or from their effect on *B. subtilis*. Butyrate causes the formation of long cell processes and induces both alkaline phosphatase and sialyltransferase needed for the formation of the sphingolipid GM_3 . In contrast to the cell process formation in Chinese hamster cells, the butyrate effect is not caused by an increase in cAMP formation. Reversion studies showed an interesting phenomenon. When cells were treated by trypsin and replated without butyrate, they transiently extended cell processes before they retracted them and resumed normal growth. If the same was done in the presence of very small concentrations of cycloheximide the retraction did not occur. Since the amount of cycloheximide needed inhibits protein synthesis only partially, the compound may have been effective in preventing the retraction of cell processes either by preferentially inhibiting protein synthesis on the cell membrane or by another mechanism independent of protein synthesis. This is under further investigation. Since a mutant of HeLa cells has been isolated which is completely resistant to the butyrate effect, the mechanism of butyrate inhibition and cell extension can now be investigated. The action of butyrate is not limited to HeLa cells because another laboratory has recently found that butyrate quite specifically induces the production of hemoglobin in Friend-cells. The action of butyrate is also worthwhile investigating because it reverses in many aspects the transformation from normal to cancer cells.

3. Characterization of viral autointerference and persistence. Many members of the myxo-, paramyxo- and rhabdovirus family can enter the central nervous system normally or as a complication of a systemic viral infection and thus cause encephalitis or meningitis. Some of these infections or their consequences seem to persist for many years. These RNA viruses have the unique property that they can produce defective particles that interfere with the multiplication of the normal virus. While this autointerference may be responsible for long-term viral persistence. The interference of normal virus development by the defective virus seems to be due to two phenomena. On the one hand, different defective viruses, containing different parts of the normal virus genome, seem to contain the same 3' and 5' end of the RNA sequence. Using one or both of these ends, they bind to the viral replicase and thereby reduce the amount of normal virus RNA that can be made. On the other hand, mRNA needed for viral protein synthesis, seems to be transcribed only from the normal virus RNA; thus the total number of viruses (infective and non-infective) into which the RNA molecules can mature is reduced owing to the interference with viral protein synthesis.

It was not possible to relate the viral persistence to the presence of virus derived DNA in the cellular chromosome. This phenomenon rather seems to depend on the continued presence of viral RNA itself, possibly kept from expressing itself owing to autointerference by defective particles. In order to measure the presence of such virus RNA molecules a new technique has been developed by which DNA complementary to the viral RNA can be produced (using reverse transcriptase. This method should be useful for the determination (by hybridization) of whether certain viral nucleic acids are actually associated with diseases such as multiple sclerosis, lupus erythematosus, or Parkinson's disease.

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| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 NS 01244-12 LMB |
| PERIOD COVERED | | |
| July 1, 1975 through June 30, 1976 | | |
| TITLE OF PROJECT (80 characters or less) | | |
| Control Mechanisms and Differentiation | | |
| NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT | | |
| PI: E. Freese Chief, Lab. of Mol. Biol. LMB NINCDS | | |
| OTHER: P. Cooney Staff Fellow LMB NINCDS | | |
| Y. Fujita Visiting Fellow LMB NINCDS | | |
| Y. Oh Staff Fellow LMB NINCDS | | |
| P. Whiteman Visiting Fellow LMB NINCDS | | |
| COOPERATING UNITS (if any) | | |
| NONE | | |
| LAB/BRANCH | | |
| Laboratory of Molecular Biology | | |
| SECTION | | |
| Developmental Biology Section | | |
| INSTITUTE AND LOCATION | | |
| NINCDS, NIH, Bethesda, Maryland 20014 | | |
| TOTAL MANYEARS: | PROFESSIONAL: | OTHER: |
| 5.4 | 4.4 | 1 |
| SUMMARY OF WORK (200 words or less - underline keywords) | | |
| <p><u>Sporulation</u> of <u>Bacillus subtilis</u> was studied as a model of <u>differentiation</u>. In contrast to the conventional assumptions of differentiation, it was shown that <u>development</u> is not irreversibly <u>triggered</u> by a single macromolecular change but rather involves the sequential formation of a number of new macromolecules rendering the differentiating cells increasingly resistant to external influences.</p> <p>It was discovered that manganese which is required for the differentiation of bacilli, exerts its effect via the activation of <u>phosphoglycerate phosphomutase</u>, an enzyme of the <u>Embden Meyerhof</u> pathway. In the absence of manganese cells accumulate 3-P-glycerate and are consequently unable to differentiate.</p> <p>It was found that bacilli can produce two different <u>glucose dehydrogenases</u>. A <u>vegetative enzyme</u> is made only when fructose-diphosphate, dihydroxy-acetone phosphate, or glyceraldehyde-3-phosphate are exhausted, i.e. the enzyme is <u>repressed</u> by carbohydrates. The developmental enzyme which is found only inside forespore cells or spores, has different specificities and other physical properties than the vegetative enzyme. Understanding the <u>control</u> of this enzyme synthesis can be useful for the production of large quantities of glucose dehydrogenase which can be easily employed for <u>glucose determination</u>.</p> | | |

Project Description:

Objectives: The developmental processes leading to cellular differentiation begin when cells stop growing and end when a genetically defined differentiated cell has formed. Our work has shown that many essential regulatory phenomena involved in differentiation are similar in micro- and higher organisms. But micro-organisms can divide faster, large numbers of identical cells can be obtained for biochemical studies, and mutants can be easily isolated. We are presently investigating differentiation in bacilli with the aim of uncovering phenomena that have general significance for all differentiation. In particular, we have investigated the assumption of a trigger of differentiation, the reason for the specific requirement of manganese for sporulation, and the control of glucose dehydrogenase as a typical developmental enzyme.

Methods Employed: Methyl-anthranilate was found to inhibit germination without affecting sporulation. It was used at a 1 mM concentration to measure the first round of sporulation observed after dilution of cells in a fresh medium, preventing the immediate germination of the newly formed spores. The accumulation of 3PGA was measured by thin-layer chromatography of ^{32}P - or ^{14}C -glycerol labeled cell extracts, or by enzymatic coupling in the direction of lactose or glycerol phosphate. Forespores were isolated by rupturing cells with a cell-mill in the presence of glass beads (100 μ in diameter in a thick paste). During shaking, the cell-mill compartment was continuously cooled.

Major Findings: 1. Absence of a trigger of differentiation. At some stage of their development cells become "committed" to continue differentiation even when they are exposed to a new environment in which they would originally have continued to duplicate. The seemingly sudden development of this commitment with respect to a given environment has created the impression that a single cellular process may irreversibly trigger the commitment with respect to all possible environments in which cells could otherwise duplicate. We have shown that this notion of a single trigger is incorrect because the developmental stage at which commitment occurs depends on the media from and to which cells are transferred. In the commitment of *B. megaterium*, it occurs earliest with respect to addition of or dilution into aspartate or glutamate, later with respect to glucose or sucrose, and latest with respect to nutrient sporulation medium. For *B. subtilis*, the formation of committed cells could be measured only in the presence of methyl-anthranilate, a compound which inhibits germination of newly formed spores, since spores otherwise germinated immediately. The sequence at which cells become committed with respect to different compounds differs in the two organisms. In *B. subtilis* commitment with respect to glucose occurs earlier than with respect to aspartate or malate; but commitment with respect to a rich medium occurs again later than that with respect to any individual compound alone. The commitment with respect to glucose and sucrose is closely correlated with a 10-fold decrease in the transport of these two compounds. In contrast, the commitment with respect to aspartate, glutamate or malate seems to result from the decrease

in an intracellular metabolic interconnection, because the transport of these compounds decreases during the developmental period only very slowly. In any case, cells become increasingly resistant to different environmental compounds owing to different biochemical changes which develop slowly and separately during the developmental period.

2. Effect of manganese on phosphoglycerate mutase and its consequences for differentiation. It was well-known that manganese is required for sporulation but not vegetative growth in a complex medium. We found that cells grow to a lower optical density in the absence than in the presence of manganese. They accumulate 3-phosphoglyceric acid (3PGA) to a 100 mM intracellular concentration in the absence of manganese. Since we had previously shown that the accumulation of organic phosphates prevents differentiation, the inability of the cells to sporulate is thus understandable. As soon as manganese is added to such a culture 3PGA disappears (is metabolized), growth resumes, and normal sporulation (S/V=80%) takes place. The accumulation of 3PGA indicated the deficiency of phosphoglycerate mutase activity. This was verified in crude extracts obtained from cells grown in the absence of manganese; phosphoglycerate mutase activity could be recovered by addition of manganese to the cell extract. The apparent K_m with respect to manganese is 0.22 mM. No other metal ions can replace manganese. The manganese dependence of phosphoglycerate phosphomutase is an unexpected finding because in all other organisms studied so far this enzyme requires either 2,3-diphosphoglycerate or no cofactor.

3. Control of glucose dehydrogenase during growth and differentiation. Glucose dehydrogenase, which converts glucose to gluconate, has been observed in bacilli only late during sporulation or within spores. We have shown in *B. subtilis* that two different glucose dehydrogenases can be produced, a vegetative and a spore-specific enzyme. The vegetative enzyme is made only when all carbohydrates are exhausted; even then it is produced during exponential growth only for a short time, namely when gluconeogenesis has not yet been initiated. Through the use of mutants blocked in different parts of the Embden Meyerhof pathway and the glycerol pathway, we have shown that the enzyme is repressed by one of three compounds: fructose-1, 6-bisphosphate, dihydroxyacetone-phosphate, or glyceraldehyde-3-phosphate. Usually, the enzyme is found only during sporulation because most growth media contain glucose or some other carbohydrate, so that the vegetative glucose dehydrogenase can be derepressed only after the end of growth at the time at which the carbohydrates have been exhausted. Toward the end of the developmental period, at the time at which a complete forespore double-membrane exists, a new glucose dehydrogenase is formed which is found only inside the forespore cell compartment and is later present in spores. In contrast to the vegetative enzyme, which is stable at all pH's and reacts only with glucose and NAD, the spore enzyme is labile at pH 8 while stable at pH 6.5, and it reacts with glucose or deoxyglucose and NAD or NADP. The physical separation of the two enzymes and the detailed characterization of their properties is under way. At present it is not known how the spore-specific glucose dehydrogenase is controlled, but it is assumed that the

closure of the forespore double-membrane creates inside the forespore a new metabolic environment which allows the derepression (or induction) of the enzyme. The characterization of these control mechanisms has also potential practical importance, because it would allow the production of large quantities of glucose dehydrogenase, an enzyme which can be easily used for glucose determinations.

Proposed Course of Project: The properties of phosphoglycerate mutase and the effect of manganese will be examined in detail and the possible manganese dependence of this enzyme in other organisms including different mammalian organs, will be determined. The two glucose dehydrogenases will be analyzed, in order to demonstrate that they are produced by different genes, and the control mechanism of the spore-specific glucose dehydrogenase will be examined in order to determine why the closure of the forespore double-membrane allows the formation of this protein. A new hypothesis has been formulated which explains why rapidly growing cells cannot differentiate and how differentiation starts. Experiments utilizing various mutants are underway to test it.

Publications:

Cooney, P.H., Freese, E.B., and Freese, E.: Commitment of Bacillus megaterium cells to sporulation, pp. 187-194. In P. Gerhardt, R.N. Costilow and H. L. Sadoff (Eds.), Spores VI, Sixth Internat'l. Spore Conference, E. Lansing, Michigan, American Society for Microbiology, 1975.

Nishihara, T., and Freese, E.: Motility of B. subtilis during growth and sporulation. J. Bacteriol., 123: 366-371, July 1975.

Cooney, P.H., and Freese, E.: Commitment to sporulation in B. megaterium and uptake of specific compounds. J. Gen. Microbiol., 1976, (in press).

Freese, E., and Fujita, Y.: Control of enzyme synthesis during growth and sporulation. Proceedings of the American Society for Microbiology Conference, April 27, 1975, (in press).

Freese, E.: Metabolic control of sporulation. Spore Group Meeting, Leeds, Great Britain, Dec. 16, 1975, (in press).

Oh, Y.K., and Freese, E.: Manganese requirement of phosphoglycerate phosphomutase and its consequence for growth and sporulation of B. subtilis. J. Bacteriol., 1976, (in press).

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| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 NS 01886-06 LMB | | | | | | | | | | |
| PERIOD COVERED July 1, 1975 through June 30, 1976 | | | | | | | | | | | | |
| TITLE OF PROJECT (80 characters or less) Developmental Cytology | | | | | | | | | | | | |
| NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT | | | | | | | | | | | | |
| <table style="width: 100%; border: none;"> <tr> <td style="width: 33%;">PI:</td> <td style="width: 33%;">E.B. Freese</td> <td style="width: 33%;">Biologist</td> <td style="width: 10%;">LMB</td> <td style="width: 10%;">NINCDS</td> </tr> <tr> <td>OTHER:</td> <td>P.H. Cooney</td> <td>Biologist</td> <td>LMB</td> <td>NINCDS</td> </tr> </table> | | | PI: | E.B. Freese | Biologist | LMB | NINCDS | OTHER: | P.H. Cooney | Biologist | LMB | NINCDS |
| PI: | E.B. Freese | Biologist | LMB | NINCDS | | | | | | | | |
| OTHER: | P.H. Cooney | Biologist | LMB | NINCDS | | | | | | | | |
| COOPERATING UNITS (if any) Univ. of Bochum, Germany Prof. P. Fortnagel, Dept. of Microbiology | | | | | | | | | | | | |
| LAB/BRANCH Laboratory of Molecular Biology | | | | | | | | | | | | |
| SECTION Developmental Biology | | | | | | | | | | | | |
| INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20014 | | | | | | | | | | | | |
| TOTAL MANYEARS: <div style="text-align: center;">2</div> | PROFESSIONAL: <div style="text-align: center;">1.7</div> | OTHER: <div style="text-align: center;">.3</div> | | | | | | | | | | |
| SUMMARY OF WORK (200 words or less - underline keywords) | | | | | | | | | | | | |
| <p>When differentiating cells are transferred to a fresh growth medium in which they would ordinarily be able to multiply, they continue their developmental process once they have reached a certain developmental stage. Using <u>sporulation</u> of <u>Bacillus subtilis</u> as a model system, it was shown by <u>electron-microscopy</u> that the morphological stage at which this commitment to continue <u>differentiation</u> occurs depends on both the original environment in which these cells multiplied and the environment in which they differentiate. The latest time of <u>commitment</u> coincides with the completion of a forespore double-membrane; i.e. a <u>membrane</u> configuration that does not permit <u>active transport</u> but only facilitated diffusion.</p> <p>Whereas cell growth is arrested by <u>fusidic acid</u>, bacilli become resistant to this <u>inhibitor</u> of <u>protein synthesis</u> at some time of <u>development</u>. The morphological stage of this resistance was identified as that of the prespore septum formation. Fusidic acid apparently interferes with <u>septation</u> as it also causes the formation of long non-separated cells.</p> | | | | | | | | | | | | |

Project Description:

Objectives: The sequential processes changing vegetatively-growing cells into differentiated cells involve biochemical and morphological changes. A correlation of these events help to understand the process of differentiation. If the differentiation process of many cells is reasonably synchronized, one can statistically determine under the electron microscope at what time, structural changes, e.g. membrane development occurs and relate it to the measurements of commitment to differentiation, to the appearance of new enzymes and to other biochemical events. Since commitment to differentiation occurs in all differentiating organisms, we have investigated this phenomenon in detail; so far, we have used *B. subtilis* in which the morphological and biochemical events leading to differentiation are better understood than in any other organism.

Methods Employed: Ultra-thin sections of bacteria were studied with the Philips Electron Microscope, EM201. The cells were fixed by glutaraldehyde fixed and osmium tetroxide, further stained uranyl acetate. After dehydration the cells were embedded in Epon, and after this sectioning with a Reichart Microtome, OMU2, stained again by lead-citrate. For each time point of sporulation development, 200-500 cells that had been longitudinally sectioned were examined to determine to which stage of asymmetric septation or membrane engulfment they had progressed. Cells were initially grown in different media, such as a nutrient sporulation medium and a minimal sporulating medium, and then during development transferred to various media containing single or multiple carbon sources. Fusidic acid was obtained from the Merck Company in Germany.

Major Findings: 1. Conditions controlling commitment of differentiation in *B. megaterium*. When cells were grown in a minimal sucrose medium, their sporulation was complete before 10 hours after the end of exponential growth (T_{10}). However, when they were transferred to a fresh minimal sucrose medium only a fraction of the cells had sporulated by T_{10} whereas the majority of cells resumed growth and sporulated later. The fraction of spores at T_{10} increased with the time at which the dilution occurred indicating that it measured the fraction of committed cells. Examination under the electron microscope showed that cells were committed before they had produced the asymmetric prespore septum. Thus, under these conditions commitment must be due to some cellular change other than the membrane alteration inside the cell; other studies in our laboratory have shown that this time is related to a drastic decrease in sucrose transport. When cells were transferred to a nutrient sporulation medium, commitment occurred at the time at which the prespore septum had been completed and the engulfment process had started. Whether this commitment is related to the completion of the septum is not yet clear. However, when cells were grown in a rich medium and then transferred to another rich medium commitment occurred at the same time at which the forespore membrane engulfment had been completed; no later commitment could be observed no matter how the medium was further enriched by other compounds. Thus, the latest commitment is apparently related to the completion

of the forespore double-membrane, which completely surrounds the forespore, thus producing a new cell compartment with different properties. The completely different behavior of this compartment with respect to enzyme synthesis and other control mechanisms can be understood because the double-membrane development shows that the two membranes have opposite polarity so that no active transport is possible through both of them (unless holes or new transport molecules are formed). Thus, compounds can reach the inner cell compartment of the forespore only by facilitated transport.

2. The effect of fusidic acid on the growth and development of *B. subtilis*. Fusidic acid is a relatively new antibiotic which inhibits protein synthesis by inhibiting the GTPase which is part of the 70S ribosome. When fusidic acid (0.5 μ M) is added to a culture of *B. subtilis*, growth stops immediately and protein and RNA synthesis are arrested. However, this compound is added during the developmental stage period, protein synthesis and sporulation continue provided the addition occurs later than T_2 . Electron microscope studies showed that the resistance to fusidate coincides with the appearance of the asymmetric prespore septum. In cell-free preparations protein synthesis also develops its resistance at about the same time indicating that the ribosomes have been altered. However, purified ribosomes do not show this alteration suggesting that the alteration can be observed only when the ribosomes are attached to membrane components. Thus, the critical event causing the resistance to fusidate seems to be the formation of the prespore septum and the corresponding membrane alteration. In fact, fusidate seems to effect membrane septation preferentially, because very low concentrations which do not inhibit protein synthesis in growing bacteria during exponential growth cause the formation of long non-dividing snakes of bacteria. Details of this membrane-associated inhibition are still under investigation.

Proposed Course of Project: An important question concerning cellular commitment is whether there is an intermediate stage between a non-committed cell able to resume growth and a committed cell obliged to continue differentiation. There are indications that cells can lose their ability to grow but are not yet committed to continue differentiation. This intermediate state will be analyzed with respect to its frequency, and media dependence; sporulation mutants blocked at later stages of development will be used to obtain cells that are all in this intermediate state. It is also intended to look at the membrane alterations related to commitment in other (nucleated) organisms.

Publications:

Freese, E.B., Cooney, P.C., and Freese, E.: Conditions controlling commitment of differentiation in *B. megaterium*. Proc. Nat'l. Acad. Sci. 72: 4037-4041, 1975.

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| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | | U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 NS 01962-05 LMB |
| PERIOD COVERED July 1, 1975 through June 30, 1976 | | | |
| TITLE OF PROJECT (80 characters or less) Mechanism and Control of Membrane Transport | | | |
| NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT PI: E. Freese, Chief, Lab. Molec. Biol., LMB NINCDS | | | |
| COOPERATING UNITS (if any) | | | |
| LAB/BRANCH Laboratory of Molecular Biology | | | |
| SECTION Developmental Biology Section | | | |
| INSTITUTE AND LOCATION NINCDS, Bethesda, Maryland 20014 | | | |
| TOTAL MANYEARS: | | PROFESSIONAL: | OTHER: |
| SUMMARY OF WORK (200 words or less - underline keywords) This project was terminated in Fiscal Year '75 but part of it has been incorporated in Project No. Z01 NS 02224-01 LMB. | | | |

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| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 NS 01963-05 LMB |
| PERIOD COVERED July 1, 1975 through June 30, 1976 | | |
| TITLE OF PROJECT (80 characters or less) Genetic Regulation in Human Diploid Tissue Culture | | |
| NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT PI: R. C. Henneberry, Senior Staff Fellow, LMB NINCDS | | |
| COOPERATING UNITS (if any) | | |
| LAB/BRANCH Laboratory of Molecular Biology | | |
| SECTION Developmental Biology Section | | |
| INSTITUTE AND LOCATION NINCDS, Bethesda, Maryland 20014 | | |
| TOTAL MANYEARS: | PROFESSIONAL: | OTHER: |
| SUMMARY OF WORK (200 words or less - underline keywords) This project was terminated in Fiscal Year '75 but part of it was incorporated in a new Project No. Z01 NS 02224-01 LMB. | | |

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| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 NS 02026-04 LMB |
| PERIOD COVERED July 1, 1975 through June 30, 1976 | | |
| TITLE OF PROJECT (80 characters or less) Regulation of Viral Nucleic Acids Synthesis in Animal Cells | | |
| NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT | | |
| PI: OTHER: | R.A. Lazzarini, Head, Molecular Virology Section, L. Johnson, Chemist S. Khan, Guest Worker J.D. Keene, Staff Fellow T. Adachi, Visiting Fellow M. Dirksen, Staff Fellow | LMB NINCDS LMB NINCDS LMB NINCDS LMB NINCDS LMB NINCDS LMB NINCDS |
| COOPERATING UNITS (if any) Laboratory of Biochemistry, NCI Serial No. NCI/LB 379 Viral Oncology Branch, NCI | | |
| LAB/BRANCH Laboratory of Molecular Biology | | |
| SECTION Molecular Virology | | |
| INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20014 | | |
| TOTAL MANYEARS: <div style="text-align: center;">4.25</div> | PROFESSIONAL: <div style="text-align: center;">3.50</div> | OTHER: <div style="text-align: center;">0.75</div> |
| SUMMARY OF WORK (200 words or less - underline keywords) | | |
| <p>The long range objective of this project is the description of the component molecular events involved in the replication of the negative strand viruses (myxo, paramyxo and rhabdo viruses). The topics that are currently being investigated are:</p> <ol style="list-style-type: none"> 1) The mechanism of <u>autointerference</u> by <u>defective interfering (DI) particles</u>. 2) The <u>nucleotide sequence</u> of <u>DI RNA</u>. 3) The mechanism of <u>viral persistency</u> and <u>carrier culture state</u>. 4) The synthesis of <u>cdna probes</u> for negative strand viruses. | | |

Project Description:

Objectives: Viral diseases of the central nervous system (CNS) usually occur as a complication rather than a normal consequence of infection. Nevertheless, many members of the myxo-, paramyxo-, rhabdovirus family, either exceptionally or as a normal consequence, infect the CNS, causing encephalitis or meningitis. Despite their importance to medical neurology, very little is known about the regulation and mode of replication of these viruses in the host organism. From what little is known, it is clear that their replication is very different from that described for polio virus or the RNA tumor viruses. Furthermore, the myxo-, paramyxo-, rhabdovirus infections also are distinguished in that they frequently elaborate defective interfering (DI) particles and exhibit evidence of autointerference and viral persistency. The description of the component molecular events involved in the replication of these viruses, the generation of defective interfering particles, autointerference and viral persistency are the subject of this project. It is anticipated that the study will delineate characteristics that can be exploited in containing and limiting viral infection to non-neural tissues or in the treatment or prevention of the viral infection.

Major Findings: 1. The mechanism of autointerference. DI particles are small viral particles that contain only a piece of the viral chromosome (often less than one gene) but all of the viral proteins. They are completely innocuous and noninfectious, but are immunologically identical to the homologous virus. When DI particles coinfect a cell with infectious particles, they severely restrict the replication of the infectious virus, a phenomenon termed autointerference. Consequently, these particles have great therapeutic potential: they can be used as a specific viral antagonist or as a vaccine to stimulate the host defense mechanisms.

We have developed a simple radiochemical procedure that is suitable for estimating the relative concentrations of both infectious and DI particles simultaneously. Using this method we have examined many of the elemental aspects of autointerference elicited by DI particles. Our results indicate that there are two central phenomena in autointerference: one influences the relative amounts of DI and full length genomes in the cell, the second determines the number of these genomes which will be matured and released from the host cell. In the first, the DI genome competes with the standard virus genome for the replicase. The relative abundance of the newly synthesized DI genome reflects its successfulness in this competition. Consequently, this competition is central in determining the proportion of the two types of genomes in the cell. Our work, however, has shown that only a small fraction of these genomes is packaged and matured into progeny particles. The second pivotal phenomenon in autointerference limits of the total yield of released virus particles both infective and defective. The limitation is imposed on the assembly of viral genomes into mature virus particles and is modulated through the availability of viral mRNA specifying the viral structural proteins. We have integrated these results into the following hypothesis. During autointerference the DI genome competes with the full length genome from the infectious particle for viral replicase. To the extent

that the DI genome is successful, the number of full length genomes in the cell diminishes. Since the full length genome is the only template for mRNA synthesis in the infected cell, the mRNA, and consequently viral proteins, become limiting at high levels of autointerference. The restricted availability of viral proteins causes a marked reduction in the total number of viral particles released from the infected cell.

2. Structure DI genomes. The ability of DI genomes to successfully compete with the full length genomes has been an enigma. VSV replication is a precise linear process: the replicating enzyme complex initiates RNA synthesis at one end of the linear template genome and moves down the template copying it. Consequently, binding sites for the replicase must be located at the termini of the template genomes. DI genomes appear to be fragments of the full length genome; some DI's contain genetic information from the left half of the infectious genome, others from the right half. How is it then that all DI's can interfere and compete with the infectious genome? One possible explanation is that both termini of the DI genomes are identical to those of the infectious particle genome. Since these termini contained the binding sites for the replicase, such a conservation of terminal sequences would account for the ability of the DI's to compete successfully for the replicase. To analyze this possibility we undertook a detailed analysis of the terminal nucleotide sequences of the genome from the infectious particle and from three dissimilar DI particles. Our results clearly show that the genomes of all the particles examined had the same sequence at the 3' end: ... PypGpU. Similarly, there appears to be conserved sequences at the 5' end of the genomes. The VSV infectious particle genome has as its 5' terminus pppApCpGp. Although the work on the DI particle genomes is not complete, our work indicates that they all terminate in pppApCpGp... also. Work is now in progress to determine if the similarity in the termini extends further into the molecules.

3. The mechanism of viral persistency. The molecular mechanisms which limit viral development and lead to virus persistency in cultured cells are obscure. Several investigators have suggested that the longevity of virus infections result from integration of the viral genome into the host chromosome. Indeed, several laboratories have presented evidence for "proviral" DNA forms of RNA viruses. We have studied viral persistency in a carrier culture of BHK₂₁ cells that have been persistently infected with VSV for over 2 years. This cell line sheds very low levels of infectious virus and DI particles, approximately one virus particle/cell/day. Nonetheless, virtually every cell in the culture produces virus proteins which are demonstrable with fluorescent antibody. We have attempted to determine if this viral persistency can be ascribed to the integration of viral information into host DNA. Two experimental approaches have been taken. First, attempts were made to transfect sensitive host cells with DNA isolated from the carrier culture. Repeated attempts at this biological identification of the viral genome have failed. Our second approach was a direct chemical identification of viral information in the DNA of the host cells. For these purposes we prepared highly radioactive DNA probe against VSV virion RNA. Using this probe we

have been unable to detect any provirus DNA copies of VSV RNA in the carrier cells. Had there been one "provirus" DNA copy per 40 cells, we would have easily detected it with this latter method. We conclude that our carrier cell culture does not contain proviral DNA and the viral persistency is due to other factors - perhaps autointerference by DI particles.

4. Synthesis of cDNA probes. Radioactive cDNA probes (DNA that is complementary to viral RNA) represent the most sensitive method for the detection and analysis of viral genomes in infected cells. Thus far it is the only way of detecting the presence of completely inactive or latent viral genetic information. We have investigated the possibility of using the reverse transcriptase obtained from RNA tumor viruses for the synthesis of cDNA. This enzyme cannot initiate DNA chains but can synthesize DNA if a primer oligonucleotide which is complementary to the template RNA is present. The enzyme extends the primer chain by the addition of deoxynucleotides and the synthesized DNA is complementary to the template RNA. We have employed a mixture of heterologous short oligonucleotides as primers for cDNA synthesis. The rationale for this approach was that short oligonucleotides can form reasonably stable duplexes with virion RNA because of the high probability that partially complementary sequences exist in the template RNA. Under appropriate conditions only one or two of these primers will bind to the template and the reverse transcriptase will begin DNA synthesis at these foci. Since there is no reason to believe that any particular region of the template should be more complementary to random fragments of DNA than any other, the probe synthesized using fragments as primers should consist of many short DNA chains which in aggregate contain all of the virion genetic information. We, together with NCI/VLL, were able to generate a probe which was complementary to vesicular stomatitis virus RNA. Preliminary studies of the DNA indicate that it has a good representation on the viral genetic information. This partially characterized probe has already been put to use in demonstrating that our persistently infected carrier culture of BHK₂₁ cells do not contain copies of the VSV genome in their DNA (described above). Our initial success with this method of synthesizing cDNA has prompted us to expand the method into a general one that can be applied to any negative strand RNA virus. If successful we will be able to detect and quantitate the presence of viral genomes in human tissues by nucleic acid hybridization. This method can be used to determine if certain suspected viral agents are indeed associated with a number of diseases of unknown etiology, e.g. measles and paramyxoviruses in multiple sclerosis; measles in systemic lupus erythematosus; influenza in post-encephalytic Parkinson's Disease.

Proposed Course of Project: 1. The further development of a general method for the synthesis of radioactive cDNA probe against negative strand RNA viruses. cDNA probes will be prepared against measles and subacute sclerosing panencephalitis (SSPE) viruses. These will be used to determine if the genetic information of these viruses can be detected in the DNA of cells undergoing persistent and slow acute viral infections with these viruses. These studies will eventually be expanded to include autopsy and biopsy tissues from patients with SSPE and systemic lupus erythematosus.

2. Further characterize the terminal of nucleotide sequences of VSV and its defective interfering particles. Attempts will be made to estimate the total length of the conserved sequences.

3. Further investigate the mechanism of autointerference. Attempts will be made to analyze the contribution of the host cell to the process of autointerference. The ability of a number of different host cells to support a normal VSV infection and the effectiveness of three standard DI particles to autointerference in these infections will be examined in detail. It is anticipated that the progeny of these infections will be enumerated with the electron microscope so that different experiments can be directly compared one with the other.

4. Our experiments with the persistently infected carrier cultures will be extended to include an analysis of the DI particles shed by these cultures. Attempts will be made to identify the contribution of autointerference to the whole process of viral persistency in cultured cells.

Publications:

Erlich, H., and Lazzarini, R.A.: Synthesis and Turnover of Ribosomal Ribonucleic Acid in Guanine-starved Cells of E. coli. J. Biol. Chem. 250: 3057-3061, 1975.

Keene, J.D., and Lazzarini, R.A.: A Comparison of the extents of Methylation of VSV messenger RNA. Virology 69: 364-367, 1976.

Lazzarini, R.A., Weber, G.H., Johnson, L.D., and Stamminger, G.M.: Covalently Linked Message and Anti-message (Genomic) RNA from a Defective VSV Particle. J. Mol. Biol. 97: 289-307, 1975.

Holland, J.J., Villarreal, L.P., Welsh, R.M., Oldstone, M.B.A., Kohne, D., Lazzarini, R., and Scolnick, E.: Long Term Persistent Rhabdovirus Infection In Vitro. J. Gen. Virol., 1976, (in press).

Orenstein, J., Johnson, L., Shelton, E., and Lazzarini, R.A.: The Shape of VSV. Virology, 1976, (in press).

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| PROJECT NUMBER (Do NOT use this space) | HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 NS 02224-01 LMB | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| PERIOD COVERED July 1, 1975 through June 30, 1976 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| TITLE OF PROJECT (80 characters or less) Cell Growth and Transport and its Inhibition by Lipophilic Acids | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| <table style="width: 100%; border: none;"> <tr> <td style="width: 10%; vertical-align: top;">PI:</td> <td style="width: 30%;">E. Freese</td> <td style="width: 30%;">Chief, Lab. Molec. Biol.,</td> <td style="width: 30%;">LMB NINCDS</td> </tr> <tr> <td></td> <td>R. C. Henneberry</td> <td>Sr. Staff Fellow,</td> <td>LMB NINCDS</td> </tr> <tr><td colspan="4"> </td></tr> <tr> <td style="vertical-align: top;">OTHER:</td> <td>E. Atikkan</td> <td>Visiting Fellow,</td> <td>LMB NINCDS</td> </tr> <tr> <td></td> <td>T. E. Iijima</td> <td>Visiting Fellow,</td> <td>LMB NINCDS</td> </tr> <tr> <td></td> <td>B. C. Levin</td> <td>Staff Fellow,</td> <td>LMB NINCDS</td> </tr> <tr> <td></td> <td>C. C. Tai</td> <td>Visiting Associate,</td> <td>LMB NINCDS</td> </tr> </table> | | | PI: | E. Freese | Chief, Lab. Molec. Biol., | LMB NINCDS | | R. C. Henneberry | Sr. Staff Fellow, | LMB NINCDS | | | | | OTHER: | E. Atikkan | Visiting Fellow, | LMB NINCDS | | T. E. Iijima | Visiting Fellow, | LMB NINCDS | | B. C. Levin | Staff Fellow, | LMB NINCDS | | C. C. Tai | Visiting Associate, | LMB NINCDS |
| PI: | E. Freese | Chief, Lab. Molec. Biol., | LMB NINCDS | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| | R. C. Henneberry | Sr. Staff Fellow, | LMB NINCDS | | | | | | | | | | | | | | | | | | | | | | | | | | | |
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| | T. E. Iijima | Visiting Fellow, | LMB NINCDS | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| | B. C. Levin | Staff Fellow, | LMB NINCDS | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| | C. C. Tai | Visiting Associate, | LMB NINCDS | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| COOPERATING UNITS (if any) Developmental and Metabolic Neurology Brnach, NINCDS Z01 NS 02164-06 DMN Georgetown University Project No. NIH N01 NS 5-2319 Johns Hopkins University Project No. NIH N01 NS 5-2320 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| LAB/BRANCH Laboratory of Molecular Biology | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| SECTION Developmental Biology Section | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20014 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| TOTAL MANYEARS: 6.5 | PROFESSIONAL: 5.5 | OTHER: 1 | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| SUMMARY OF WORK (200 words or less - underline keywords) <p>The goal of this project is to understand the mechanisms controlling growth and membrane transport and their inhibition by <u>lipophilic acids</u>, which include most anti-microbial <u>food additives</u>, <u>cosmetic preservatives</u>, <u>antiseptics</u>, <u>certain pesticides</u>, and various <u>drugs</u>. Some of the highly lipophilic compounds are known or suspected to cause brain damage or to be teratogens. The reactions of <u>Bacillus subtilis</u>, for which mutants and vesicles of cytoplasmic membranes can be easily obtained, are compared with <u>HeLa</u> and other mammalian cell lines which can be more directly related to the mammalian problem. Results include: (1) The process formation of HeLa cells induced by butyrate and the reversion to a normal cell type were studied. (2) <u>Hexachlorophene</u> inhibits growth by destroying the proton gradient through the cell membrane. Almost all of it is bound to the cells and cannot be removed by washing. However, exposure to <u>bovine serum albumin</u> removes it and restores growth. (3) <u>B. subtilis</u> can grow on <u>aspartate</u> as sole carbon source even when cells lack the (high affinity) <u>aspartate transport</u> system; this growth is <u>sodium</u> dependent.</p> | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |

Project Description:

Objectives: Lipophilic acids have been used for many decades as preservatives, antiseptics, pesticides and drugs. Until recently the cellular mechanisms of most of these compounds remained unknown. Work in this laboratory, started under Project No. Z01 NS 01962-04 LMB, has shown that anti-microbial food additives and certain antiseptics inhibit bacterial growth by destroying the proton gradient across the cell membrane thus starving the cells. When the same and other compounds were examined for their effect on tissue cultures, the same extent of inhibition was usually found, with the exception of some compounds, i.e. propionate, butyrate, and analgesics, which caused inhibition at a much lower concentration, indicating a specific cellular effect. The cause of these types of growth inhibition in mammalian cells is as yet unknown but it clearly increases with the lipophilicity of the compounds used. Some highly lipophilic compounds are increasingly found to represent environmental hazards to man causing teratogenic effects, brain damage in children, and tremors, skin eruptions or sterility in adults. Our intent is to study on the cellular level how these phenomena are produced, how the detrimental effects of lipophilic compounds can be avoided, and once a person has been exposed how he can be treated.

In addition to mammalian cells, we investigate the effect of these compounds on *Bacillus subtilis* as a simple model system because these bacteria have essentially the same sensitivity to lipophilic acids as mammalian cells, but results are obtained five times faster and with higher statistical accuracy, and mutants can be employed in order to examine a particular hypothesis. As a consequence of work started under Project No. Z01 NS 01963-04 LMB the system of glutamate and aspartate transport have been singled out for this mutant investigation. Both a low and a high affinity transport system have been found and growth at high concentrations of aspartate is sodium dependent similar to the sodium dependence of transport observed in mammalian cells.

As a by-product of these studies, we have found that straight chain fatty acids of low molecular weight, in particular butyrate, inhibits epithelial cells more potently than could have been expected from their partition coefficient or their inhibitory effect on bacteria. Butyrate also produces neurite-like processes and induces certain enzymes and alters membrane components. These alterations represent the reversal of the changes ordinarily observed when normal cells are transformed into cancer cells by, e.g., viruses. Butyrate can also affect differentiation because another laboratory reported recently that butyrate induces the formation of hemoglobin Friend cells. For these reasons we are investigating the effect of butyrate and its reversal on cultured mammalian cells.

Methods Employed: The presence of the butyrate-induced protein necessary for the formation of neurite-like processes can be determined by trypsinizing the cells and plating them in the presence of cycloheximide which prevents the resumption of growth and the retraction of cell processes. Mutants of HeLa cells resistant to inhibition by butyrate have been isolated by the ability to grow in the presence of butyrate. ¹⁴C-hexachlorophene was

used to measure the binding of this compound to cells. Since hexachlorophene also binds to membrane filters, the cells were centrifuged and the amount of ^{14}C was measured in the pellet and in the supernatant. Oxygen consumption was measured by polarography. Mutants of *Bacillus subtilis* deficient in the high affinity transport of aspartate or glutamate were isolated by their resistance to threo-hydroxy-aspartate. Their genetic location was mapped by transduction with PBS1 phage.

Major Findings: 1) Effect of butyrate on HeLa cells. Previous studies had shown that many lipophilic acids inhibit HeLa cells to the same extent as they inhibit *Bacillus subtilis*, but that propionate, butyrate and pentanoate are inhibitory at a much lower concentration and simultaneously cause the formation of neurite-like processes. This process formation was a reversion of cancer-like properties to a more normal cell type, i.e. the opposite of what is frequently observed as a transformation of tissue cultures. Since such transformation often involves the conversion of a lipopolysaccharide to a simple type (containing fewer carbohydrate residues), it appeared possible that the opposite conversion might be induced by butyrate. In fact, the lipopolysaccharide (Cer-glc-gal) was converted to one containing a sialic acid (G_{M3}) residue, as was measured in collaboration with the Developmental and Metabolic Neurology Branch, NINCDS. It was also found the corresponding enzyme (sialyltransferase I) was induced about twenty-fold by butyrate.

When the reversion of the butyrate (5 mM) induced processes was studied after butyrate removal, the trypsinized cells reextended their processes dramatically and the original morphology reappeared only slowly. The reversion was blocked by surprisingly low concentrations of cycloheximide (0.5 $\mu\text{g/ml}$), suggesting a specific effect of this compound; either it inhibited protein synthesis only in the cell membrane or it had another effect independent of protein synthesis.

Fatty acid resistant variants of HeLa cells and a mutant completely resistant to 5 mM butyrate were isolated spontaneously or after chemical mutagenesis. These mutants will be used to examine the effect of butyrate, in particular its biochemical specificity. We have demonstrated that butyrate effects are not influenced by the presence of exogenous cyclic AMP or its analogs and that they are not mediated by intracellular cyclic AMP: The concentration of intracellular cyclic AMP increases in the presence of both butyrate and decanoate whereas only the former causes the characteristic shape changes. Both butyrate and decanoate also cause changes in the surface glycopeptides but again these effects are not correlated with the morphological differentiation. The last aspect of the project is more fully described in Project No. Z01 NS 02164-06 DMN from the Developmental and Metabolic Neurology Branch, NINCDS.

2) Comparison of the effects of hexachlorophene and decanoate on *Bacillus subtilis* and reversion by bovine serum albumin. Hexachlorophene is a highly lipophilic antimicrobial agent which was extensively used as an antiseptic until it was shown to cause brain damage. Many other highly lipophilic compounds (DDT, PCB, Kepone, etc.), which are used as pesticides or in other ways, apparently can also effect the human nervous system and thus constitute potential environmental hazards. We have compared its effect

on bacteria with that of decanoate, which is much less lipophilic. Both compounds inhibit bacterial growth by destroying the proton gradient across the cell membrane (uncoupling) as was demonstrated by the fact that they inhibit amino acid transport but do not inhibit the oxidation of glycerol phosphate or NADH in membrane preparations. Whereas the effect of decanoate is reversible upon dilution or washing of the bacteria, that of hexachlorophene is irreversible under these conditions giving the impression that the bacteria are dead. The reason for this irreversibility was shown to be the extraordinary strong binding of hexachlorophene to the cells, 80% of the decanoate compound being bound. The bound hexachlorophene could not be washed off. In contrast, less than 1% of the decanoate was bound to cells and even that could be removed by washing. The binding studies showed that roughly 5×10^5 molecules of hexachlorophene and 100 times as many molecules of decanoate per *B. subtilis* cell were needed to prevent cell duplication. The much higher number of decanoate molecules suggests that most of this compound is located in the cytoplasm rather than on the cell membranes. Since many lipophilic drugs adsorb to serum albumin, the effect of this compound on hexachlorophene binding was measured. Addition of 1% bovine serum albumin to a cell-suspension removed more than 93% of the bound hexachlorophene and immediately restored growth. Our results indicate a method by which other highly lipophilic compounds, which may enter humans via the skin or otherwise but cannot leave again via the urine, may be removed. These compounds probably establish an equilibrium between the albumin in the blood and the cells or proteins in other organs. Therefore, people inadvertently exposed to highly lipophilic toxic compounds could be treated by repeated replacement of their serum albumin, for example, by plasmapheresis or washing of blood with activated charcoal. Obviously, one wants to avoid the appearance of such toxic problems which could be achieved by washing the skin of exposed persons with suspensions of lipophilic polymers.

3) Properties of aspartate transport mutants. In order to analyze the growth and transport properties of mutants deficient in glutamate or aspartate transport, mutants resistant to three-hydroxy-aspartate were isolated. These mutants lacked the high affinity transport of both glutamate and aspartate but the transport of all other amino acids were unimpaired. The mutation was mapped to be between *argC* and *glpD* in the *B. subtilis* map. To our surprise both the normal and the mutant strains, could grow on high concentrations of aspartate as sole carbon source and this growth required the presence of sodium. Transport studies demonstrated that in addition to the high affinity transport, there is a low affinity transport system functioning only at very high aspartate concentrations; it can be detected in whole cells but not in membrane vesicles. Whether this low affinity transport system or some metabolic step utilizing aspartate is sodium dependent is still under investigation.

Proposed Course of Project: The mechanism by which lipophilic acids inhibit the growth of mammalian cells will be investigated because it relates to the mechanism of action of many environmental compounds and drugs. In particular, the effect of butyrate will be examined because it is very specific and produces neurite-like cell processes. We have also found that

the effect of antiseptics and other lipophilic acids on cells depends to some extent on the growth medium. Since this phenomenon may explain the inefficiency of some of these compounds in certain environments and may provide another tool for treatment after toxic exposure, its biochemical origin will be examined. The investigation of the aspartate and glutamate transport system serves as a tool for the mutant analysis of transport alterations; its sodium dependence may represent an analog of the sodium dependence of mammalian substrate transport which needs investigation.

Publications:

Simmons, J.L., Fishman, P.H., Freese, E., and Brady, R.O.: Morphological alterations and ganglioside sialyltransferase activity induced by small fatty acids in HeLa cells. J. Cell Biol., 66: 414-424, 1975.

Fishman, P.H., Bradley, R.M., and Henneberry, R.C.: Butyrate-induced glycolipid biosynthesis in HeLa cells: Properties of the induced sialyltransferase. Arch. Biochem. Biophys., 172: 618-626, 1976.

Henneberry, R.C., and Fishman, P.: Morphological and biochemical differentiation in HeLa cells: An unusual effect of cycloheximide on process formation and ganglioside metabolism. J. Exp. Res., (in press).

Appendix: Georgetown University Project No. NIH N01 NS 5-2319 and Johns Hopkins University Project No. NIH N01 NS 5-2320.

Fifty lipophilic acids about half of them known to be teratogenic and half of them suspected to be teratogenic owing to their molecular structure were investigated. On the one hand their pK values, partition coefficients and distribution coefficients were determined in order to predict their lipophilic tendency at a given pH. Some compounds were so lipophilic that they had to be dissolved in 50% or more ethanol and their pK value had to be calculated from the titration curve correcting for the effect of ethanol. On the other hand, the inhibition of HeLa cell growth was measured at pH 7.2 and at different concentrations of the compounds. Where possible the compounds were dissolved in alkaline solution or directly in the medium so that no other organic materials were added. Occasionally, ethanol had to be used because of the high lipophilicity of the compound; but the final ethanol concentration was always less than 1%. Compounds with especially high inhibitory potency were, for example, chlorambucil, dicoumarol, hexachlorophene, retionic acid, and salicylanilid. The results indicate a good correlation between the distribution coefficient and the inhibitory potency of a compound and suggest that these properties in turn may determine the teratogenic potency of the compounds. This will have to be verified in the future.

ANNUAL REPORT
July 1, 1975 through June 30, 1976
Laboratory of Neuro-otolaryngology, IRP
National Institute of Neurological and
Communicative Disorders and Stroke

Jörgen Fex, M.D., Ph.D., Chief

During Fiscal Year 1976 all major renovations and installments of equipment in the space assigned to the Laboratory were completed and all personnel slots were filled.

The Laboratory is staffed and equipped for an advanced multidisciplinary approach to biological research; it is in this respect unique in the field of auditory research. This uniqueness permits a productive team work within the Laboratory in the disciplines of biochemistry, morphology, pharmacology and physiology. Using techniques from all these disciplines, and serving the purpose of the Laboratory to conduct research that will give new knowledge on biological factors underlying normal and abnormal hearing, the Laboratory now focuses on the normal inner ear and cochlear nucleus of mammalian species; research has also been initiated on the biochemistry of the inner ear and cochlear nucleus of genetically deaf animals.

Through its research on the morphology and biochemistry of the inner ear the Laboratory is already on the forefront of auditory research and of research in general on enzymes and synapses. In particular, during this fiscal year, the Laboratory contributed with the following new knowledge, in the press or in manuscripts under preparation.

A major cochlear enzyme, a true cochlear carbonic anhydrase not due to cochlear blood, was isolated and purified. This enzyme constituted about 1% of the total protein of the cochlear membranous wall lateral to the endolymphatic space. The high concentration of the enzyme justified the new hypothesis that carbonic anhydrase has an important function for maintaining the electrolyte concentrations in endolymph, high for potassium and low for sodium.

Methods for micro-assays of enzymes in cochlea and cochlear nucleus were further developed. Levels of activity and distributions in the guinea pig cochlea and cochlear nucleus of enzymes that catalyze the synthesis of putative transmitter substances were determined. The levels of choline acetyltransferase (ChAc) in different parts of the organ of Corti corresponded to elsewhere described distribution of olivocochlear nerve fibers and endings; the findings added evidence to the hypothesis that olivocochlear neurons are cholinergic. Glutamate decarboxylase (GAD) was low in the cochlea; this new finding was strong evidence against a recent hypothesis that GABA is a cochlear transmitter substance. ChAc, GAD and tyrosine hydroxylase were low in the auditory nerve and did not decrease in the cochlear nucleus after surgical destruction of the cochlea; this permitted the conclusion that none of acetylcholine, GABA or the catecholamines is a major transmitter substance for the auditory nerve in the cochlear nucleus.

The junctions of the membranes of the auditory sensory cells and adjoining supporting cells were studied under the electron microscope using thin sections and the freeze-fracture technique. Tight junctions were found, including a variation of junctions not described before. These junctions were considered to form the structural basis for the maintenance of the large ionic differences between endolymph and perilymph, without which there is no auditory function. Using similar electron microscopic techniques, synapses of the organ of Corti were studied. Both pre- and postsynaptic membrane structures at inner hair cells were different from those at outer hair cells, providing new evidence for the hypothesis that the different hair cells may use different transmitter substances. Also, it was established that there are no gap junctions across hair cell synaptic clefts; electrical synaptic transmission in the traditional sense was therefore excluded as a means of excitation in the organ of Corti. However, the presynaptic structure and organization of the outer hair cell membrane were not identical with those of chemical synapses yet described.

There are no immediate plans to change the focus of the Laboratory; the two Projects of the Laboratory will be advanced. Thus, during the Fiscal Year 1977, physiological experiments will be initiated that include sound stimulation and recording of activity of the auditory nerve and of cells in the cochlear nucleus. This is planned to lead to a long-range study on activity of cochlear cells that are activated by electrical stimulation and sound and to which putative transmitter substances and their antagonists are electrophoretically applied.



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| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 NS 02147 02 LNO |
| PERIOD COVERED July 1, 1975 to June 30, 1976 | | |
| TITLE OF PROJECT (80 characters or less) Central Connections of the Auditory Efferent System | | |
| NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT PI: J. C. Adams Staff Fellow LNO NINCDS | | |
| COOPERATING UNITS (if any) None | | |
| LAB/BRANCH Laboratory of Neuro-otolaryngology | | |
| SECTION | | |
| INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20014 | | |
| TOTAL MANYEARS: 0 | PROFESSIONAL: 0 | OTHER: 0 |
| SUMMARY OF WORK (200 words or less - underline keywords) During FY 1976 this project was incorporated with Project No. Z01 NS 02217 01 LNO. | | |

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| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 NS 02148 02 LNO |
| PERIOD COVERED July 1, 1975 to June 30, 1976 | | |
| TITLE OF PROJECT (80 characters or less) Isolation and Characterization of Cochlear Transport Proteins | | |
| NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT PI: D. G. Drescher Senior Staff Fellow LNO NINCDS | | |
| COOPERATING UNITS (if any) None | | |
| LAB/BRANCH Laboratory of Neuro-otolaryngology | | |
| SECTION | | |
| INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20014 | | |
| TOTAL MANYEARS: 0 | PROFESSIONAL: 0 | OTHER: 0 |
| SUMMARY OF WORK (200 words or less - underline keywords) During FY 1976 this project was incorporated with Project No. Z01 NS 02216 01 LNO. | | |

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| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 NS 02149 02 LNO |
| PERIOD COVERED July 1, 1975 to June 30, 1976 | | |
| TITLE OF PROJECT (80 characters or less) A Morphological Study of the Junctions of the Inner Ear, Including the Synapses | | |
| NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT <div style="display: flex; justify-content: space-between;"> <div style="width: 30%;"> PI: R. L. Gulley OTHER: T. S. Reese </div> <div style="width: 35%; text-align: center;"> Senior Staff Fellow Head, Section on Functional Neuro- anatomy </div> <div style="width: 30%; text-align: right;"> LNO NINCDS LNNS NINCDS </div> </div> | | |
| COOPERATING UNITS (if any) None | | |
| LAB/BRANCH Laboratory of Neuro-otolaryngology SECTION | | |
| INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20014 | | |
| TOTAL MANYEARS: <div style="text-align: center;">0</div> | PROFESSIONAL: <div style="text-align: center;">0</div> | OTHER: <div style="text-align: center;">0</div> |
| SUMMARY OF WORK (200 words or less - underline keywords) During FY 1976 this project was incorporated with Project No. Z01 NS 02216 01 LNO. | | |

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| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 NS 02216 01 LNO | | | | | | | | | | | | | | | | | | | | |
| PERIOD COVERED July 1, 1975 to June 30, 1976 | | | | | | | | | | | | | | | | | | | | | | |
| TITLE OF PROJECT (80 characters or less) Inner Ear Neuronal Mechanisms: A Multidisciplinary Analysis Incorporating Project Nos. Z01 NS 02146 02 LNO, Z01 NS 02148 02 LNO, and Z01 NS 02149 02 LNO. | | | | | | | | | | | | | | | | | | | | | | |
| NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT | | | | | | | | | | | | | | | | | | | | | | |
| <table style="width: 100%; border: none;"> <tr> <td style="width: 15%; vertical-align: top;">PI:</td> <td style="width: 35%;">J. Fex</td> <td style="width: 35%;">Chief, LNO</td> <td style="width: 15%;">LNO NINCDS</td> </tr> <tr> <td></td> <td>D. G. Drescher</td> <td>Senior Staff Fellow</td> <td>LNO NINCDS</td> </tr> <tr> <td></td> <td>R. L. Gulley</td> <td>Senior Staff Fellow</td> <td>LNO NINCDS</td> </tr> <tr> <td></td> <td>R. J. Wenthold</td> <td>Staff Fellow</td> <td>LNO NINCDS</td> </tr> <tr> <td style="vertical-align: top;">OTHER:</td> <td>T. S. Reese</td> <td>Head, Section on Functional Neuro- anatomy</td> <td>LNNS NINCDS</td> </tr> </table> | | | PI: | J. Fex | Chief, LNO | LNO NINCDS | | D. G. Drescher | Senior Staff Fellow | LNO NINCDS | | R. L. Gulley | Senior Staff Fellow | LNO NINCDS | | R. J. Wenthold | Staff Fellow | LNO NINCDS | OTHER: | T. S. Reese | Head, Section on Functional Neuro- anatomy | LNNS NINCDS |
| PI: | J. Fex | Chief, LNO | LNO NINCDS | | | | | | | | | | | | | | | | | | | |
| | D. G. Drescher | Senior Staff Fellow | LNO NINCDS | | | | | | | | | | | | | | | | | | | |
| | R. L. Gulley | Senior Staff Fellow | LNO NINCDS | | | | | | | | | | | | | | | | | | | |
| | R. J. Wenthold | Staff Fellow | LNO NINCDS | | | | | | | | | | | | | | | | | | | |
| OTHER: | T. S. Reese | Head, Section on Functional Neuro- anatomy | LNNS NINCDS | | | | | | | | | | | | | | | | | | | |
| COOPERATING UNITS (if any) | | | | | | | | | | | | | | | | | | | | | | |
| D. K. Morest, Harvard Medical School, Boston, Massachusetts 02115 | | | | | | | | | | | | | | | | | | | | | | |
| LAB/BRANCH Laboratory of Neuro-otolaryngology | | | | | | | | | | | | | | | | | | | | | | |
| SECTION | | | | | | | | | | | | | | | | | | | | | | |
| INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20014 | | | | | | | | | | | | | | | | | | | | | | |
| TOTAL MANYEARS: <div style="text-align: center;">7.2</div> | PROFESSIONAL: <div style="text-align: center;">3.3</div> | OTHER: <div style="text-align: center;">3.9</div> | | | | | | | | | | | | | | | | | | | | |
| SUMMARY OF WORK (200 words or less - underline keywords) | | | | | | | | | | | | | | | | | | | | | | |
| <p>The long range purpose of the project is to study the biochemistry, morphology, pharmacology and physiology of inner ear neurons and cells and of their interactions and to describe the mechanisms behind these interactions.</p> <p>The interest is now focused on: 1) the isolation and characterization of <u>cochlear</u> transport proteins, which has led to the purification of <u>carbonic anhydrase</u> from the cochlear membranous lateral wall; 2) the ultrastructure of <u>junctions</u> of the inner ear, including synapses, as seen under the <u>electron microscope</u> in thin sections and in freeze-fracture replicas; 3) the levels of <u>choline acetyltransferase</u> (ChAc) and <u>glutamate decarboxylase</u> (GAD) in the cochlea, with findings fitting the hypothesis that the <u>olivocochlear nerve fibers</u> are cholinergic and that GABA is unlikely to be a transmitter in the organ of Corti; 4) the <u>in vitro uptake</u> of <u>amino acids</u> (glutamic acid, aspartic acid, GABA, glycine and leucine in the inner ear of guinea pig); 5) the levels of choline acetyltransferase in the normally <u>developing inner ear</u> and in the inner ear of a genetically deaf mutant (the <u>waltzing guinea pig</u>).</p> | | | | | | | | | | | | | | | | | | | | | | |

Project Description:

Objectives: To study the biochemistry, morphology, pharmacology and physiology of inner ear neurons and cells and of their interactions and to describe the mechanisms behind these interactions.

The following subprojects are now serving these objectives:

- I. Isolation and characterization of cochlear transport proteins.
- II. A morphological study of the junctions of the inner ear, including the synapses.
- III. A biochemical study of synaptic transmission in the inner ear.
- IV. An autoradiographic study of synaptic transmission in the inner ear.
- V. A biochemical study of the inner ear of genetically deaf animals.

Methods Employed:

I. Cochlear membranous lateral walls (stria vascularis, spiral ligament, spiral prominence, and part of the outer sulcus) from guinea pigs were dissected at 2°C in isotonic medium and homogenized. Cochlear carbonic anhydrase was purified by gel filtration and ion exchange chromatography and the purification was monitored by polyacrylamide electrophoresis. The enzyme assay incorporated a colorimetric method for determining the pH drop accompanying CO₂ hydration. Protein was analyzed by standard visible and UV spectrophotometry. In a comparative study using similar methods, carbonic anhydrases B and C from guinea pig blood were also purified to homogeneity.

II. The cochleas of chinchilla were fixed by simultaneous perfusion of the perilymphatic space and vascular system. The organ of Corti was fixed with various combinations of aldehyde fixatives, washed in buffered glycerine and frozen in Freon at -146°C and stored in liquid nitrogen. The tissue was fractured and replicated on a Balzers' 360 M freeze-fracture unit. The replicas were examined with the electron microscope. Additional animals were fixed with aldehydes, postfixed in OsO₄, stained en bloc with uranyl acetate and embedded in Araldite. Thin sections from this tissue were examined with the electron microscope. The extracellular tracers, horseradish peroxidase and lanthanum, were used to study the permeability of the junctional structures in the organ of Corti.

III. Sensitive micro assays capable of detecting enzymatic activity in a few micrograms of tissue were developed. A technique for dissecting the organ of Corti in guinea pig was developed. Activities of choline acetyltransferase (ChAc) and glutamate decarboxylase (GAD), enzymes catalyzing the synthesis of acetylcholine (ACh) and gamma-aminobutyric acid (GABA), respectively, were measured in the cochlea of the guinea pig.

IV. Guinea pig cochleae were dissected out, the bony shell was removed under fluid. The remaining specimen, including the spiraling organ of Corti was incubated for 20 min in a solution of one amino acid at 1-2 μM in 120 mM NaCl, 5 mM KCl, 10 mM glucose, 20 mM NaPO_4 , 1.3 μM MgSO_4 at a final pH of 7.4. The amino acids used were: Glutamic Acid [$3 - ^3\text{H}$] 23.4 Ci/mmole; Aspartic Acid [$2,3 - ^3\text{H}$] 17.8 Ci/mmole; GABA [$2,3 - ^3\text{H}$] 39.22 Ci/mmole; Glycine [$2 - ^3\text{H}$] 9.39 Ci/mmole; Leucine [$4,5 - ^3\text{H}$] 50 Ci/mmole. All amino acids were purified by high voltage electrophoresis using pyridine/acetic acid, pH 3.95. The spirals were immersed for two hours in fixative containing 3% glutaraldehyde, 2% paraformaldehyde in 0.1 M sodium cacodylate with 20 mM CaCl_2 . The fixative was changed three times during the first nine minutes. Then the spiral was divided into four turns in 0.2 M sodium cacodylate with 20 mM CaCl_2 , postfixed in 2% OsO_4 in 0.1 M sodium cacodylate and 20 mM CaCl_2 , dehydrated in a graded series of methanol and embedded in Araldite. At least 30 semi-serial thick sections ($\sim 1 \mu\text{M}$) were cut from each turn of the cochlea, dipped in Kodak NTB emulsion, and stored at 4°C for 21 days. The slides were then developed in Dektol, washed and fixed in Kodak-Rapidfix, washed and stained with Richardson's stain.

V. The methods described above, for III, were used for determining the levels of choline acetyltransferase in the organ of Corti in developing animals from age three days and upwards, using normal guinea pigs and the genetically deaf mutant (the waltzing guinea pig).

Major Findings:

I. Conclusive evidence has been found for a true cochlear carbonic anhydrase not due to cochlear blood. One major cochlear enzyme, similar in charge to blood enzyme C, has been isolated; thus an inner-ear enzyme has been purified for the first time. About 1% of the protein of the cochlear membranous lateral wall is carbonic anhydrase. Acetazolamide produces half-maximal inhibition of the enzyme at a concentration near 10^{-8}M .

II. The results fall into three groups: 1) tight and gap junctional structures, 2) non-junctional membrane specializations of the hair cells and supporting cells, and 3) synapses.

1) The apices of the cells in the reticular lamina are joined by a band of tight junctions spaced at 140 Å intervals. Beneath this apical band the organization of the tight junctions depends on whether they join a supporting cell and a hair cell, or two supporting cells. At hair cell junctions with supporting cells, there is an extensive labyrinth of tight junctions enclosing lengthy, tortuous passages. At appositions between two supporting cells, maculae or fasciae occludentes lie beneath the apical bands of closely spaced tight junctions, near the top of the zonulae adherentia which are characteristic of appositions between supporting cells. The complexes of tight junctions between extralaminar supporting cells differs from those in the reticular lamina. The extralaminar cells are joined by a band of four to seven branching, anastomotic tight junctions. Thus, these junctions are like zonulae

occludentes in other tissues. The organization of the tight junctions in the reticular lamina, different from those between the extralaminar supporting cells, suggests a special role for these junctions in the reticular lamina. Two sizes of gap junctions link, and presumably couple, supporting cells in the reticular lamina.

2) Freeze-fractured hair cells have on their lateral borders unique membrane specializations, not associated with intercellular junctions. Inner hair cells had two characteristic specializations on the cytoplasmic leaflets of their plasma membrane; single rows of large particles interspersed with typical membrane particles and large clusters of smaller particles packed in rectilinear arrays with a 90 Å spacing. Both specializations were found opposite expanses of extracellular space as well as supporting cells, so neither can be part of an intercellular junction. Comparable regions of the lateral membranes of outer hair cells also had a characteristic structure. The cytoplasmic membrane leaflet was covered with loosely packed large particles, while the external membrane leaflet had scattered groups of particles on it. These particles also occurred on regions of the membrane not lying over the lateral cisterns. In the synaptic region, at the base of the outer hair cell, perturbations of the otherwise smooth surface of the external membrane leaflet formed cross-hatched patterns over appositions with the afferents and opposite expanses of extracellular space, but not over appositions with efferents. This distribution is comparable with that of a small cistern lying near the hair cell plasmalemma, which are distinct from the subsurface cisterns under efferent terminals as well as the lateral cisterns described above. The cytoplasmic leaflets of their plasma membrane of supporting cells, where they faced the basilar membrane, were covered with orthogonal assemblies of small particles, which characterize astrocytes in the central nervous system and are thought to be important in cellular transport.

III. ChAc activity in the organ of Corti, third turn, was 1270 pmole ACh formed/min/mg protein (ChAc, 1270) and was higher than in turn 4 (ChAc, 543). ChAc activity was higher when the preparation included the inner hair cell region than when not. GAD activity in samples of turn 3 and 4 combined was low, 0.17 nmole GABA formed/min/mg protein (GAD, 0.17). The findings on the distribution of ChAc activity in the organ of Corti fit the hypothesis that the olivocochlear nerve fibers are cholinergic. Because of low GAD in the cochlea, GABA is unlikely to be a transmitter in the organ of Corti.

IV and V. These subprojects are in their beginning stages with no major findings.

Significance to Biomedical Research and the Program of the Institute: This multidisciplinary study on inner ear neurons and cells and their interactions provides new knowledge on the poorly understood inner ear mechanisms of hearing. Such new knowledge is of direct significance to biomedical research, will lead to better understanding of the causes of sensory deafness and nerve deafness and will most likely lead to better management of hearing disorders.

In particular, referring to subprojects: I) Carbonic anhydrase probably controls the endolymphatic concentration of electrolytes, thus indirectly providing the bias for how hair cells transform acoustical energy into electrochemical energy. The study of this enzyme should add to our understanding of the transducer function of the hair cells. It is an important result that it has been purified from the cochlea. II) An increased knowledge of the inner ear synapses is badly needed for biomedical research on the inner ear. This study has already provided the important information that there are no electrical synapses between hair cells and auditory neurons and has also clarified how the electrolyte barrier between endolymph and the organ of Corti is being upheld. III and IV) The transmitter substance(s) between hair cells and auditory nerve fibers is(are) unknown. The present studies have made earlier suggestions that GABA be such a transmitter untenable. The studies have also added to earlier evidence that the cochlear efferent nerve fibers are cholinergic. The studies will continue to forward knowledge on inner ear transmitter substances; such knowledge is important for the understanding of hearing processes in the normal and in the diseased ear. V) The waltzing guinea pig is an animal model for the study of hereditary deafness. This study (along with a subproject of Project No. Z01 NS 02217 01 LNO) is designed to biochemically characterize specific proteins in the cochlea (respectively, the cochlear nucleus) of the waltzing guinea pig. It is the initial effort in an investigation of hereditary deafness at the molecular level.

Proposed Course: The biochemical study of synaptic transmission in the inner ear (subproject III) is temporarily halted; its further course will be guided partly by the findings of our study on amino acid uptake in the organ of Corti. The other subprojects will be continued as follows.

I. The carbonic anhydrase from cochlea will be further characterized by physicochemical means, and compared to other carbonic anhydrases. Distribution of the enzyme in cochlear tissues will be determined. Measurement of K^+ and Na^+ in endolymph before and after administration of acetazolamide is planned.

II. The studies will be continued to relate membrane specializations to synaptic functions in the organ of Corti.

IV. The autoradiographically prepared sections of the organ of Corti after amino acid uptake will be inspected; the findings will lead to a publication, perhaps after similar experiments have been added. Other studies of the organ of Corti, using radioactive tracers, are being planned.

V. The study of the levels of choline acetyltransferase in the normally developing cochlea and in the cochlea of the waltzing guinea pig will be continued. Studies of other proteins in the waltzing guinea pig are planned.

Publications:

I. Drescher, D. G.: A review of general cochlear biochemistry in normal and noise exposed ears. In Henderson, D., Hamernik, R. P., Dosanjh, D. S., and Mills, J. H. (Eds.): The Effects of Noise on Hearing. New York, Raven Press, 1976. In press.

II. Gulley, R. L., and Reese, T. S.: Intercellular junctions in the reticular lamina of the organ of Corti. J. Neurocytol. In press.

III. Fex, J., and Wenthold, R. J.: Choline acetyltransferase, glutamate decarboxylase and tyrosine hydroxylase in the cochlea and cochlear nucleus of the guinea pig. Brain Res. In press.

IV and V: None

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| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 NS 02217 01 LNO | | | | | | | | | |
| PERIOD COVERED | | | | | | | | | | | |
| July 1, 1975 to June 30, 1976 | | | | | | | | | | | |
| TITLE OF PROJECT (80 characters or less) | | | | | | | | | | | |
| Synaptic Transmission and Neuronal Connections of the Mammalian Cochlear Nucleus | | | | | | | | | | | |
| Incorporating Project Nos. Z01 NS 02146 02 LNO and Z01 NS 02147 02 LNO | | | | | | | | | | | |
| NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT | | | | | | | | | | | |
| <table border="0"> <tr> <td>PI: J. Fex</td> <td>Chief, LNO</td> <td>LNO NINCDS</td> </tr> <tr> <td>J. C. Adams</td> <td>Staff Fellow</td> <td>LNO NINCDS</td> </tr> <tr> <td>R. J. Wenthold</td> <td>Staff Fellow</td> <td>LNO NINCDS</td> </tr> </table> | | | PI: J. Fex | Chief, LNO | LNO NINCDS | J. C. Adams | Staff Fellow | LNO NINCDS | R. J. Wenthold | Staff Fellow | LNO NINCDS |
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| Laboratory of Neuro-otolaryngology | | | | | | | | | | | |
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| INSTITUTE AND LOCATION | | | | | | | | | | | |
| NINCDS, NIH, Bethesda, Maryland 20014 | | | | | | | | | | | |
| TOTAL MANYEARS: | PROFESSIONAL: | OTHER: | | | | | | | | | |
| 4.6 | 1.8 | 2.8 | | | | | | | | | |
| SUMMARY OF WORK (200 words or less - underline keywords) | | | | | | | | | | | |
| <p>The long-range purpose of the project is to study the biochemistry, morphology, pharmacology and physiology of synaptic transmission and neuronal connections of nerve cells in the mammalian cochlear nucleus. The interest is now focused on: 1) the development of a system of <u>computer programs</u> for storing and displaying information on the localization, synaptic connections and activity of cells in the cochlear nucleus and for <u>controlling experiments</u> and processing results; 2) the levels of <u>choline acetyltransferase</u> (ChAc), <u>glutamate decarboxylase</u> (GAD) and <u>tyrosine hydroxylase</u> (TH) in the auditory nerve and the cochlear nucleus of the normal <u>guinea pig</u>; 3) the levels of ChAc, GAD, TH and of putative transmitter substances (<u>amino acids</u> and <u>peptides</u>) in the guinea pig cochlear nucleus after surgical destruction of the cochlea; 4) the levels of ChAc and of GAD in the normally developing cochlear nucleus and in the cochlear nucleus of a genetically deaf mutant (the <u>waltzing guinea pig</u>); 5) the levels of ChAc and of GAD in anatomically defined subgroups of the guinea pig cochlear nucleus. New findings indicate that it is unlikely that <u>acetylcholine</u>, <u>GABA</u> or a catecholamine is a major transmitter between the auditory nerve and the cochlear nucleus.</p> | | | | | | | | | | | |

Project Description:

Objectives: To study the biochemistry, morphology, pharmacology and physiology of synaptic transmission and neuronal connections of nerve cells in the mammalian cochlear nucleus. The following subprojects are now serving these objectives,

- I. A physiological study of synaptic transmission in the cochlear nucleus.
- II. A biochemical study of synaptic transmission in the cochlear nucleus.
- III. A biochemical study of the cochlear nucleus of genetically deaf animals.
- IV. Central connections of the auditory efferent system.

Methods Employed:

I. A system of computer programs is being developed for storing and displaying information on the localization, synaptic connections and activity of cells in the cochlear nucleus and for controlling experiments and processing results.

II. Sensitive micro assays capable of detecting enzymatic activity in a few micrograms of tissue were used. Amino acids were determined using an amino acid analyzer. Three groups of guinea pigs were used. 1) The auditory nerve and the cochlear nucleus were analyzed for levels of choline acetyltransferase (ChAc), glutamate decarboxylase (GAD) and tyrosine hydroxylase (TH). 2) The guinea pig cochlea on one side was surgically destroyed. The levels of ChAc, GAD and TH and of putative transmitter substances (amino acids) in the cochlear nucleus bilaterally (unoperated side as control) were determined at 2, 4 and 6 weeks after surgery. 3) The cochlear nucleus was removed and subdivided under the microscope in anatomically defined subgroups; the levels of ChAc and GAD in each subgroup were determined.

III. The levels of ChAc and GAD were determined in the cochlear nucleus of developing animals from age three days and upwards, using normal guinea pigs and a genetically deaf mutant (the waltzing guinea pig).

IV. In cats, cells were labeled following injection of horseradish peroxidase into the inferior colliculus. Refinements of this technique were developed which include intensification of the reaction product by pretreatment of tissue with cobalt chloride and combining the enzymatic labeling with silver impregnations.

Major Findings:

- I. The subproject is in its beginning stage with no major findings.
- II. ChAc, GAD, and TH were all low in the auditory nerve of the normal guinea pig and the level of neither of these enzymes decreased in the cochlear nucleus following surgical ablation. These new findings indicate that it is unlikely that acetylcholine, GABA or a catecholamine is a major transmitter between the auditory nerve and the cochlear nucleus.
- III. The subproject is in its beginning stage with no major findings.
- IV. Descending projections to the inferior colliculus were found to originate in layer IV of the auditory cortex and in thalamic nuclei, including the peripeduncular nucleus, the suprapeduncular nucleus, the medial division of the medial geniculate body, and the parabrachial nuclei. Ascending projections to the inferior colliculus were revealed in the same experiments. These projections include bilateral input from the cochlear nucleus, the medial superior olive, the lateral superior olive, the periolivary cell groups, and the dorsal and ventral nuclei of the lateral lemniscus. Only a minority of cells giving rise to these projections had been previously identified.

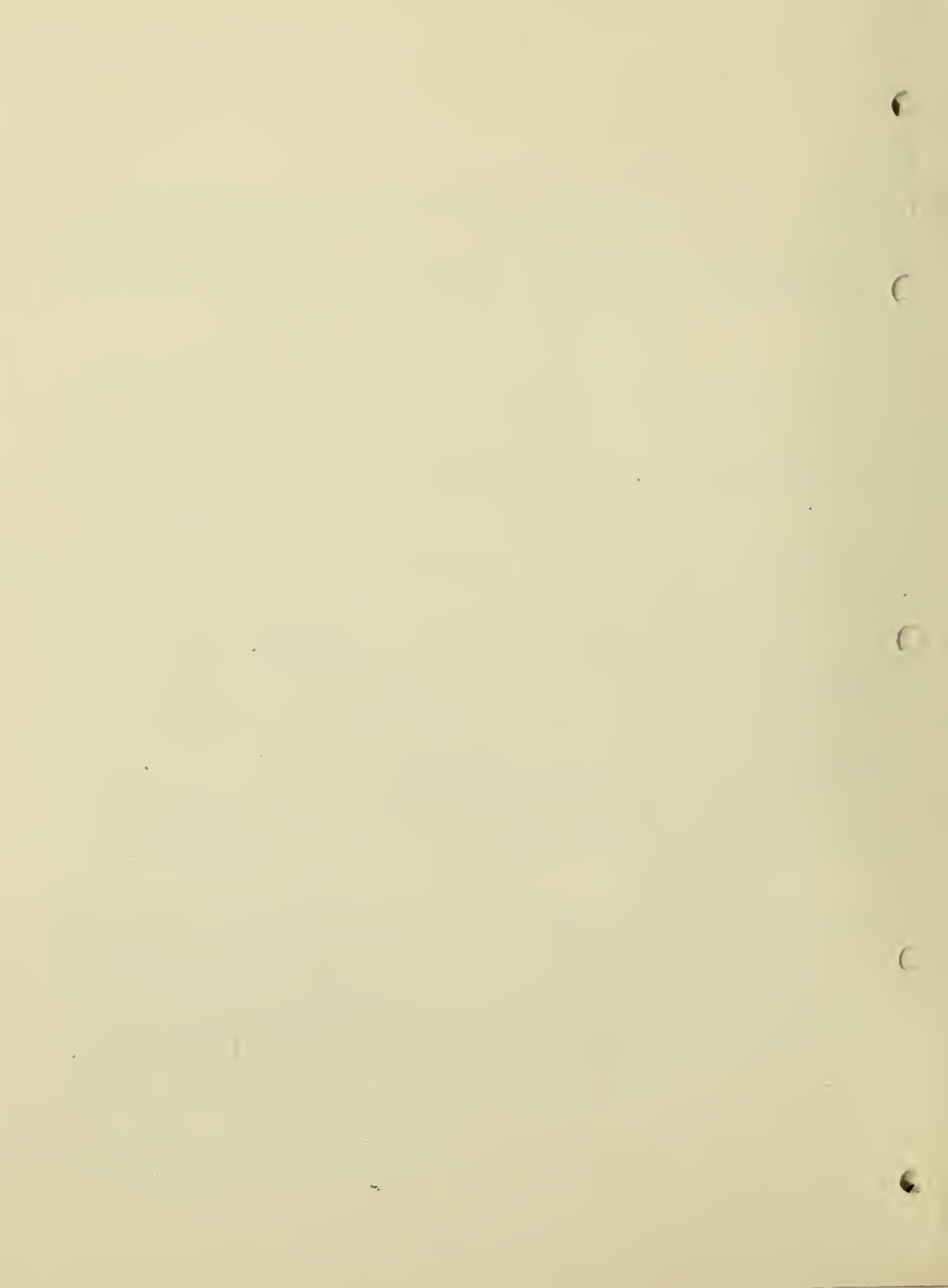
Significance to Biomedical Research and the Program of the Institute:

This multidisciplinary study on synaptic transmission and neuronal connections of the mammalian cochlear nucleus provides new knowledge on how nerve impulses in the auditory nerve are decoded at the first relay station for transmission to higher levels of the central auditory nervous system. Any such knowledge will be of importance for the program of the Institute if an auditory prosthesis for implantation in the auditory nerve or the cochlear nucleus is to be developed. Our biochemical study of the cochlear nucleus of the genetically deaf guinea pig is (along with a subproject of Project No. Z01 NS 02216 01 LNO) the initial effort in an investigation of hereditary deafness at the molecular level.

Proposed Course:

I. Starting during Fiscal Year 1977, the physiology and pharmacology of cells in the cochlear nucleus will be studied with the use of electrophoretic application of putative transmitter substances and their blockers during simultaneous recording of single unit activity, extracellularly and intracellularly. The choice of cells to be recorded from and of substances to be applied will depend on the results of subprojects II, III, and IV. The course of subprojects II and III will depend on results that are now being collected and analyzed. Subproject IV will continue, focusing on the central connections of the cochlear nucleus.

Publications: None



ANNUAL REPORT
July 1, 1975 through June 30, 1976
Laboratory of Neuropharmacology
National Institute of Neurological and
Communicative Disorders and Stroke

The Laboratory of Neuropharmacology has, in its second year of operation, continued basic and clinically applied studies of central synaptic mechanisms, with the goal of extending our ability to pharmacologically modify central neuronal function in order to provide more effective therapies for neurologic disease. During the past year, Laboratory staffing has been completed with individuals whose strengths lie in complementary clinical and preclinical areas. A vertically integrated structure has thus been developed to facilitate translation of basic information into improved therapeutic strategies and to promote the rapid preclinical testing of hypotheses deriving from clinical studies. Research operations, conducted in largely completed facilities in Buildings 10 and 36, continued to focus on monoaminergic and GABA containing neuronal systems, especially in relation to extrapyramidal function. In addition, transfer of the Unit on Stroke from the Extramural Research Program has permitted an extension of investigative activities to include pharmacologic aspects of cerebrovascular disease. The Laboratory's scientific achievements are detailed in five Project Reports and have resulted in 40 journal articles or book chapters published or accepted for publication during the year.

I. GABA System

Although involuntary movements occurring in patients with Huntington's disease have long been attributed to degeneration of striatal interneurons, recent observations suggest that a loss of GABA-containing striatal efferents to the substantia nigra and globus pallidus contribute to the motor disorder. Accordingly, the Laboratory has continued to explore rational approaches to the pharmacologic manipulation of GABA-mediated synaptic function. One aspect of this work has involved studies of mechanisms regulating GABA synthesis. Using a newly developed, highly sensitive procedure for estimating the activity of glutamic acid decarboxylase (GAD), the rate limiting enzyme in GABA synthesis, the relationship between the GAD apoenzyme and its cofactor, pyridoxal phosphate, has been studied in microdissected regions of rat brain. Available results indicate that GAD in vivo may be at most 60% saturated with cofactor and that certain endogenous substances reduce this binding to 40% or less. GAD activity thus may be regulated, at least partially, by factors which limit the availability of pyridoxal phosphate to the apoenzyme. These investigations have also demonstrated that classical kinetic analyses used to describe the relationship between GAD and pyridoxal phosphate are not valid for assessing changes in the affinity between GAD and its cofactor.

Related experiments have provided some basis for the possibility that manipulatable differences exist between GAD in striatal efferent systems and GAD in other GABA-containing neuronal pathways. This possibility derives from the observation that there are substantial regional differences in GABA turnover.

For example, substantia nigra and globus pallidus have relatively slow synthesis rates for GABA compared with the cingulate, parietal or entorhinal cortex; pyriform and cerebellar cortex have intermediate GABA turnover rates. Brain regions with faster rates of GABA turnover appear more sensitive to a depression in GABA synthesis induced by drugs such as barbiturate or benzodiazepine derivatives, which presumably act either directly or indirectly to stimulate GABA receptors.

Evidence in support of the hypothesis that the GABA-containing striato-nigral pathway influences the activity of the nigro-striatal dopamine system has also been obtained. Blockade of GABA receptors by picrotoxin substantially increases impulse activity of some dopaminergic neurons in the pars compacta of substantia nigra. Moreover, drug induced elevations in brain GABA were found to attenuate the ability of haloperidol to accelerate striatal dopamine synthesis. This attenuation was blocked by picrotoxin. These results suggest that the GABA pathway may exert a tonic inhibitory effect on certain dopaminergic neurons arising in the substantia nigra, and that the ability of antipsychotic agents such as haloperidol to stimulate dopamine turnover may in part be mediated by the GABA system. On the other hand, elevations in brain GABA failed to alter impulse activity in nigral dopamine cells of otherwise untreated rodents. These latter observations indicate that pharmacologic attempts to increase brain GABA concentrations may not necessarily augment GABA mediated synaptic transmission, and may explain why drugs which alter GABA levels are ineffective in the symptomatic relief of neurologic disorders attributable to the degeneration of GABA-containing neurons.

II. CSF Studies

A newly assembled primate facility has enabled the extension of *in vitro* neurochemical observations to the integrated waking primate and the validation of new approaches to the clinical study of neuropharmacologic aspects of central nervous system function. Chair restrained, waking, rhesus monkeys, adapted to preset environmental and dietary conditions, have shown characteristic variations in the central turnover of dopamine, norepinephrine and serotonin as estimated by levels of their principle metabolites in ventricular fluid. Circadian variations in these neurotransmitter metabolites were generally coincident with changes in CSF concentrations of cyclic AMP as well as with body activity and brain temperature. Future investigations will employ drugs to further explore the relationship between CSF levels of neurotransmitters or their metabolites, the activity of specific central neurohumoral systems, and various behavioral parameters.

Studies have recently been initiated on the use of stable (nonradioactive) isotopes for the evaluation of central neurotransmitter metabolism in man. This approach has important potential advantages over existing methodology which combine either radioisotope labeling techniques or probenecid loading strategies with estimations of transmitter metabolites in lumbar spinal fluid. Preliminary results indicate that Oxygen 18 (given in a breathing mixture containing 95% O₁₈) as well as deuterated methionine (given intravenously) provide sufficient labelling of CSF catecholamine metabolites in primates to allow reliable turnover estimates. Toxicologic evaluation of O₁₈ in rodents revealed no adverse effects and thus permission has now been sought for the

clinical use of 018.

III. Extrapyramidal Disease

Clinical studies of the pharmacodynamic characteristics and therapeutic potential of a new class of dopamine agonists have expanded during the past year. These investigations now indicate that the ergot derivative, bromocriptine, exceeds L-dopa in therapeutic efficacy in patients with idiopathic Parkinson's disease. Rigidity, tremor and facial expression showed the greatest response. Adverse effects were transient and dose dependent. Although the severe "on and off" effects which plague the response of parkinsonian patients to L-dopa also occur with bromocriptine, the frequency of this complication is significantly reduced. The administration of caffeine to parkinsonian patients failed to potentiate the therapeutic effects of bromocriptine, despite the potent ability of this phosphodiesterase inhibitor to enhance the motor effects of L-dopa or dopamine receptor agonist in animal models of parkinsonism.

Based on a recent report that another dopamine receptor agonist, apomorphine, alleviates involuntary movements in Huntington's chorea patients, the therapeutic effects of bromocriptine were also investigated in this disorder. A double blind crossover study showed, however, that bromocriptine exacerbated choreatic movements, thus supporting the contention that hyperkinesia in Huntingtonian patients may reflect hyperactivity of the dopamine system.

The chronic administration of therapeutic dose levels of bromocriptine to parkinsonian or Huntington's chorea patients lead to significant reductions in CSF levels of the dopamine metabolite, homovanillic acid. In contrast, probenecid induced accumulations of 5-hydroxyindoleacetic acid were substantially increased, suggesting that an increase in serotonin turnover attends bromocriptine therapy. There was no change in CSF cyclic AMP levels. Bromocriptine also produced a significant rise in growth hormone levels in patients with Huntington's chorea but not in those with Parkinson's disease. Circulating prolactin concentrations were reduced by bromocriptine in both groups of patients. The lack of growth hormone response to bromocriptine in Parkinsonian patients may reflect an attenuation in dopamine receptor sensitivity.

Recent reports of diminished choline-acetyl transferase activity in patients with Huntington's chorea suggest that some degenerating striatal neurons in this disorder may use acetylcholine as their neurotransmitter and that pharmacologic attempts to restore cholinergic function might afford symptomatic relief. Although therapeutic trials of physostigmine have yielded generally disappointing results, cholinesterase inhibitors might be expected to have relatively little effect in situations where cholinergic dysfunction arises as a consequence of the loss of neurons which synthesize and release this neurotransmitter. Under such circumstance, advantages may accrue from the administration of drugs, such as pilocarpine, which directly stimulate postsynaptic cholinergic receptors and thus are independent of cholinergic terminals for pharmacologic activity. Unfortunately, however, patients with Huntington's chorea given a therapeutic trial of high doses of pilocarpine showed no significant improvement in neurologic function. Since this result may reflect the fact that pilocarpine is a relatively weak cholinergic agonist, preclinic

toxicological studies of more potent stimulators of postsynaptic cholinergic receptors have now been initiated.

IV. Lead Poisoning

Preclinical and clinical studies initiated during the past year on the pathogenesis of lead neurotoxicity have focused on the role of central dopaminergic mechanisms. In vitro experiments have demonstrated that incubation of brain minces with lead significantly increases the release of dopamine and inhibits the reuptake of this neurotransmitter. Clinical observations have corroborated these laboratory results by showing that undue lead exposure in children is associated with an increased urinary excretion of the catecholamine metabolites, homovanillic acid and vanillylmandelic acid. Although no clear correlation was found between the degree of homovanillic acid elevation and other biochemical indicators of lead exposure, the excretion of this dopamine metabolite tended to return to normal during effective chelation therapy.

V. Cerebrovascular Disease

Anesthetic agents including barbiturate derivatives reportedly exert a protective effect on neuronal tissues during ischemic episodes. In order to further explore this possibility, mongolian gerbils received unilateral carotid artery ligation during either barbiturate or cyclohexylamine (ketamine) anesthesia. The yield of infarcted cerebral hemispheres was substantially lower in barbiturate treated gerbils. These results support the contention that drugs which are believed to diminish cerebral metabolic demands, may limit tissue damage due to vascular occlusion and thus possibly be of value to some patients with cerebral vascular disease.

Recent studies have sought to improve noninvasive techniques for distinguishing completed strokes from transient ischemic attacks. Such methods assume special importance in situations where ischemic damage has occurred in clinically silent areas of the nervous system or in patients under consideration for microsurgical revascularization procedures. Investigations carried out during the past year indicate that acute focal cerebral ischemia induced in primates by segmental middle cerebral artery occlusion produces a prompt rise in cisternal CSF levels of the neuronal isoenzyme of creatine phosphokinase (CPK). No concomitant alteration in this isoenzyme was observed in venous blood, nor were values for the muscle isoenzyme of CPK significantly changed. Attempts to correlate the degree of enzyme elevation with the volume of cerebral infarction are currently in progress.

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| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER <div style="text-align: center; font-weight: bold;">Z01 NS 02139-02 LP</div> |
| PERIOD COVERED <div style="text-align: center;">July 1, 1975 to June 30, 1976</div> | | |
| TITLE OF PROJECT (80 characters or less) <div style="text-align: center;">Pharmacology, biochemistry and physiology of central neurotransmitters</div> | | |
| NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT | | |
| PI: Judith R. Walters Thomas N. Chase | Staff Fellow Chief | LP/NINCDS LP/NINCDS |
| OI: Leonard Miller Danka Pericic Ellen Silbergeld Ronald Kartzinel David Martin | Staff Fellow Guest Worker Staff Fellow Clinical Associate Associate Professor | LP/NINCDS LP/NINCDS LP/NINCDS LP/NINCDS Univ. Md. |
| COOPERATING UNITS (if any) <div style="text-align: center;">University of Maryland, Department of Chemistry, College Park, Maryland</div> | | |
| LAB/BRANCH <div style="text-align: center;">Laboratory of Neuropharmacology</div> | | |
| SECTION <div style="text-align: center;">None</div> | | |
| INSTITUTE AND LOCATION <div style="text-align: center;">NINCDS, NIH, Bethesda, Maryland 20014</div> | | |
| TOTAL MANYEARS: <div style="text-align: center;">6.4</div> | PROFESSIONAL: <div style="text-align: center;">3.8</div> | OTHER: <div style="text-align: center;">2.6</div> |
| SUMMARY OF WORK (200 words or less - underline keywords) | | |
| <p>The purpose of this project is to improve our understanding of ways in which drugs may alter centrally mediated neurotransmission and to develop better pharmacotherapies for neurological disorders. Topics currently under investigation include: (1) Factors regulating <u>glutamic acid decarboxylase</u> activity; (2) Effects of drugs on γ-aminobutyric acid (GABA) synthesis; (3) Role of GABA-containing neurons in the regulation of <u>dopaminergic</u> function; (4) Neurotransmitter dysfunction in Huntington's chorea and (5) Use of the stable isotope of oxygen (O_{18}) to evaluate central <u>monoamine metabolism</u> in man.</p> | | |

Project Description:

Objectives: To improve understanding of the process of central neurotransmission, especially as mediated by γ -aminobutyric acid (GABA) and dopamine (DA), to examine the effects of drugs on various aspects of this process, and to apply such knowledge towards the rational development of more effective pharmacologic treatments for nervous system disease.

I. Regulation of GABA synthesis

In order to investigate approaches to the pharmacologic manipulation of GABA-mediated transmission, the Laboratory has been (a) studying the mechanisms which control glutamic acid decarboxylase (GAD) activity in various regions of rat brain and (b) evaluating techniques for estimating rates of GABA synthesis in vivo in the presence and absence of drugs.

Methods Employed: These included analysis of enzyme activity in homogenates, dialysates or precipitates of brain tissue and microfluorometric assay of GABA levels in microdissected brain regions from control or drug treated animals.

Major Findings and Significance to Biomedical Research:

(a) GAD activity

Investigations of factors controlling the activity of GAD, the rate-limiting enzyme in GABA synthesis, have focused on the interaction between the GAD apoenzyme and its cofactor, pyridoxal phosphate (PLP). In vitro studies have shown that the cofactor can become tightly bound to the enzyme relatively rapidly, but is released in the presence of glutamic acid. A mathematical equation has been developed to describe these interactions between cofactor and apoenzyme. By varying the conditions used during brain homogenization, it has been possible to estimate that GAD in vivo may be, at most, 60% saturated with cofactor. Moreover, preliminary results now indicate that certain endogenous substances reduce this binding to 40% or less. Thus it appears that GAD activity in vivo may be determined, at least in part, by elements which limit the availability and binding of PLP to the apoenzyme. These investigations have also demonstrated that the classical enzyme kinetic analyses previously used by other workers to describe the relationship between GAD and PLP are not valid for assessing changes in the affinity between GAD and its cofactor.

A new column assay procedure which is appropriate for determination of GAD activity in a single rat substantia nigra has been developed to obtain baseline information on GAD activity in soluble, membrane bound and whole nigral (triton-solubilized) fractions. Contrary to some indications obtained from previous whole brain studies with the CO₂ trapping assay (and contrary to observations made with tyrosine hydroxylase, another rate-limiting enzyme in transmitter synthesis), the pH optimum, Km's and the activity of GAD in the absence of substrate did not vary in these different fractions.

(b) GABA turnover

GABA turnover has been estimated by following the accumulation of this amino acid in specific brain regions after the administration of aminooxyacetic acid (AOAA), a drug which preferentially inhibits the catabolizing enzyme, GABA transaminase (GABA-T). Although it appears that this technique cannot provide estimates of absolute GABA synthesis rates because GABA-T is not completely inhibited by sublethal doses of AOAA, and because GAD activity in some areas appears decreased by AOAA, nevertheless, this technique is the best that is currently available. Studies to date indicate that the relative synthesis of GABA is slower in the substantia nigra and globus pallidus than in the cingulate, parietal and entorhinal cortexes; pyriform and cerebellar cortex and the caudate nucleus have intermediate GABA turnover rates.

GABA levels and relative synthesis rates have been investigated in the basal ganglia and cerebral cortex of rats treated with various drugs which appear to modify GABA-mediated neurotransmission. Diazepam, which is thought to increase the stimulation of GABA receptors, decreased the apparent turnover of GABA in the caudate nucleus, parietal, cingulate and entorhinal cortex, but not in the substantia nigra, globus pallidus, pyriform and cerebellar cortex. Blockade of GABA receptors with picrotoxin increased GABA accumulation in some regions but did not attenuate the depressant effect of the benzodiazepines on GABA turnover. This latter observation casts doubt upon the idea that the decrease in GABA turnover caused by diazepam is a compensatory response elicited by the effect of the benzodiazepine on GABA receptors. Barbiturates elevated GABA levels in the caudate nucleus but, even in sub-anesthetic doses, depressed GABA turnover in some cortical regions. γ -Hydroxybutyric acid (GHB) completely inhibited the accumulation of GABA after AOAA. The GHB-induced inhibition of GABA turnover was not blocked by picrotoxin, a finding which does not support the idea that GHB is a GABA agonist. In these studies, GHB was active in doses as low as are required to cause significant changes in the DA system, suggesting that GHB does not interact preferentially with DA neurons in the CNS. Chloral hydrate, an anesthetic which has not been linked to changes in GABA function, also decreased the accumulation of GABA after AOAA but was less effective in this regard than GHB or barbiturates.

It is not yet clear whether the drugs influence the apparent rate of GABA synthesis by direct effects on impulse activity in GABA neurons or by interacting with enzymes or precursor pools controlling GABA synthesis. It was found that brain regions exhibiting faster rates of GABA turnover, also show a greater depression of GABA synthesis with the drugs tested. These observations suggest that certain biochemical or neurophysiological attributes of GABA containing neuronal systems possess substantial regional variations, which may provide a means of achieving pharmacological selectivity in the manipulation of GABA-mediated function.

The hypothesis that a GABAergic striato-nigral feedback loop controls the activity of the nigro-striatal DA neurons was investigated by testing the effects of various neuroleptics on GABA levels and turnover. It was found that dopaminergic receptor blockers do not change GABA levels, but they significantly enhance GABA accumulation after administration of AOAA in the substantia nigra and pyriform cortex. Clozapine and chlorpromazine were more active in this regard than haloperidol. There were also differences in the activity of these drugs on GABA accumulation from region to region. These findings do not correlate with the relative effects of neuroleptics on DA neurons but they do suggest that the neuroleptics also may affect the turnover of GABA.

II. Effect of GABAergic and dopaminergic neurotransmission on the activity of cells in the extrapyramidal system

Methods Employed: Studies utilize (1) determinations of single unit activity of DA cells in the substantia nigra of anesthetized rats, and (2) estimation of drug-induced changes in the apparent *in vivo* synthesis rate of DA by measurement of DA precursor and metabolite levels in brain homogenates.

Major Findings and Significance to Biomedical Research:

(a) Effects of GABA on neuronal activity

In order to further evaluate the possibility of pharmacologically altering GABA-mediated neuronal transmission, the activity of a population of cells which are thought to be innervated by GABA terminals, i.e., the DA cells in the pars compacta of substantia nigra, is being investigated after administration of various agents which might increase or decrease stimulation of GABA receptors.

Elevation of GABA levels in the substantia nigra did not alter single unit activity of DA cells in the pars compacta. On the other hand, picrotoxin, a GABA receptor blocker, increases the firing rate of a portion of the A9 DA cell population. These results are in agreement with apparent changes in DA synthesis noted after administration of AOAA or picrotoxin. Because a strio-nigral projection using GABA as a transmitter is thought to modify the firing rate of DA cells when drugs affecting DA-mediated transmission in the striatum are administered, the ability of AOAA or picrotoxin to modify the effects of amphetamine and haloperidol on dopaminergic activity is being investigated. AOAA was shown to attenuate the effect of amphetamine, a drug thought to increase striatal DA release. This observation does not support the idea that a neuronal pathway using GABA as an inhibitory transmitter mediates the inhibitory effects of amphetamine on dopaminergic activity. Moreover, picrotoxin failed to block the effects of amphetamine on the activity of DA neurons.

AOAA, on the other hand, attenuated the effect of haloperidol on the synthesis of striatal DA. This attenuation was blocked by picrotoxin, suggesting it was mediated by GABA neurons and that the elevation of GABA levels following AOAA may counteract some of the inhibitory effects of haloperidol on the putative

GABAergic striato-nigral pathway. The activation of DA synthesis caused by haloperidol does not appear to be due solely to the ability of haloperidol to inhibit the activity of a GABAergic striato-nigral pathway, however, because blockade of GABA receptors in the substantia nigra with picrotoxin causes a much smaller change in DA synthesis rates than does haloperidol.

(b) Effects of DA on neuronal activity

A project has recently been initiated in the Laboratory to investigate the possibility that DA may be released from processes in the substantia nigra. Direct injection of DA receptor blocking drugs into the substantia nigra produces behavioral changes which suggest that DA receptors in this region are being tonically stimulated.

III. Studies on neurotransmitter dysfunction in patients with extrapyramidal disease

Methods Employed : Clinical studies are conducted in patients with neurological disorders and in normal volunteers. Under basal conditions and during the administration of drugs believed to influence central synaptic mechanisms, neurologic and psychologic parameters of brain function are correlated with results of (a) chemical assay of relevant compounds in various body fluids and tissues, (b) isotopic tracer procedures and (c) electrical measures of spontaneous and evoked cortical potentials.

Major Findings and Significance to Biomedical Research:

(a) Pilocarpine in Huntington's chorea

An attempt was made to directly stimulate acetylcholine receptors in the brain of patients with Huntington's chorea using a cholinomimetic drug (pilocarpine). Peripheral parasympathomimetic actions of pilocarpine were antagonized by an antimuscarinic agent (methscopolamine) given concurrently. Five patients were studied in a double-blind investigation, the maximum doses of pilocarpine being 35 mg as a single dose. There was no significant change in clinical evaluations, thus casting doubt on hypothesis that degeneration of cholinergic neurons, as suggested by the reduction on choline acetyltransferase activity in the striatum of Huntington's patients, contributes to the choreatic movements occurring with the disorder.

(b) CSF Studies with Probenecid

Probenecid blocks the active transport from CSF to blood of homovanillic acid and 5-hydroxyindoleacetic acid, thus increasing CSF levels of these products of central monoamine metabolism. The rate of increase provides an index to the central turnover of the parent amines. During the past few years, probenecid has been administered orally to study patients, although nausea and vomiting not infrequently ensues and undoubtedly effects the gastrointestinal absorption of the drug. In an attempt to mitigate these difficulties, clinical studies

have been conducted in collaboration with NIMH using intravenously administered probenecid. The half-life in plasma of probenecid given as a single intravenous infusion (40 mg/kg) to patients with either Huntington's chorea or Parkinson's disease averaged about 6.6 hours. In CSF, peak values for homovanillic acid and 5-hydroxyindoleacetic acid occurred in samples collected 8 hours after beginning the 1 hour probenecid infusion. Even after 4 hours, however, levels of both monoamine metabolites were significantly increased. There was a positive correlation between CSF levels of probenecid and the increase in 5-hydroxyindoleacetic acid but not homovanillic acid. Compared with the oral administration of probenecid the intravenous infusion technique produced more consistent elevation in plasma and CSF probenecid levels, greater increases in CSF homovanillic acid values, and fewer gastrointestinal side-effects.

(c) CSF Studies with O_{18}

In the continuing search for improved biochemical methods to evaluate the functional status of specific neurohumoral systems in the central nervous system of man, recent efforts have focused on the possible use of stable (non-radioactive) isotopes. The systemic administration of the stable isotope of oxygen, O_{18} , to various animal species has now been shown to produce adequate labeling of brain and CSF monoamines, and their principle metabolites. Toxicologic studies have now also been completed in both rats and baboons given prolonged exposure to a breathing mixture containing 95% O_{18} . No adverse effects were observed by the usual chemical or histologic criteria, and thus these studies will now proceed to humans.

Proposed Course:

Investigation of factors regulating GABA synthesis will continue. The effects of drugs and different behavioral states on the in vivo saturation of GAD by PLP will be investigated further, and in vitro systems for studying the effects of drugs on GABA synthesis will be explored. Single unit recording techniques will be applied to further investigation of the interaction of GABA and DA-containing neurons. Techniques permitting monitoring of single unit activity during extracellular iontophoresis of drugs and neuroactive substances are being developed to further our understanding of these systems. Clinical studies during the coming year will focus on the therapeutic trials of drugs such as clozapine believed to relatively selectively block postsynaptic dopaminergic receptors in the mesolimbic system and on the application of inspired O_{18} to evaluate the central metabolism of monoamines and other neurotransmitters.

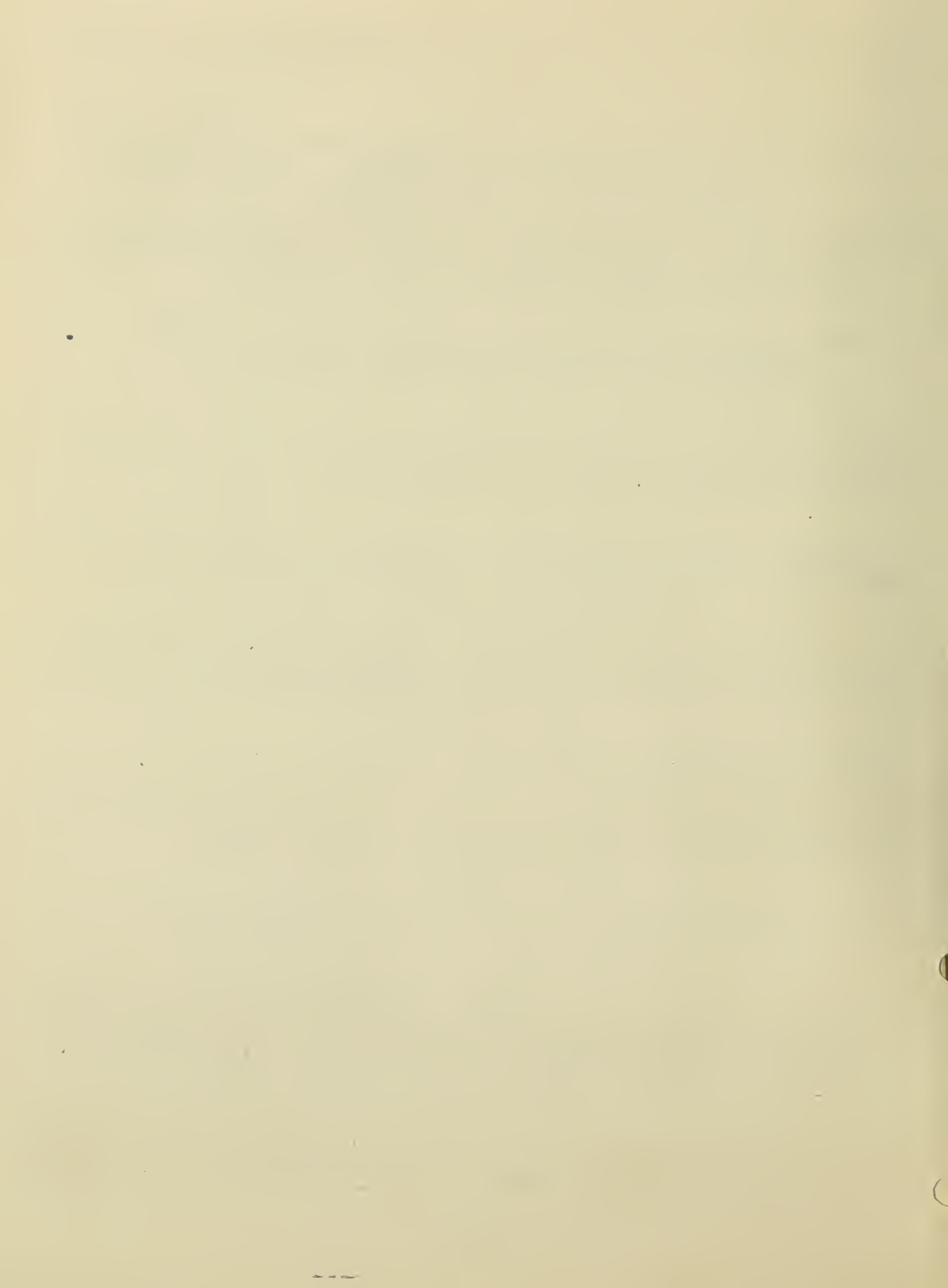
Publications:

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2. Shoulson, I. and Chase, T.N.: Caffeine and the antiparkinsonian response to levodopa or piribedil. Neurology 25:722-724, 1975.
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23. Shetty, T. and Chase, T.N.: Central monoamine and hyperkinesia of childhood. Neurology, in press.
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PERIOD COVERED

July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Studies with Bromocriptine in Extrapyramidal Disease

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

| | | |
|-----------------------|---------------------|-----------------------|
| PI: Donald B. Calne | Clinical Director | OD/NINCDS |
| OI: Ronald Kartzinell | Clinical Associate | LP/NINCDS |
| Paul Teychenne | Visit. Assoc. | LP/NINCDS |
| Ira Shoulson | Resident in Neurol. | Univ. Roch. Sch. Med. |
| Robert Hunt | Clinical Associate | LCS/NIMH |
| Thomas N. Chase | Chief | LP/NINCDS |
| Ann Carter | Prof. of Medicine | Downstate Med. Ctr. |
| Christy Ludlow | Speech Therapist | CDP/NINCDS |
| Mark Perlow | Staff Fellow | LP/NINCDS |
| Andrea Gielen | Psychologist | OD/NINCDS |
| Marge Gillespie | Nurse | OD/NINCDS |
| Ron Pfeiffer | Guest Worker | OD/NINCDS |

COOPERATING UNITS (if any)

National Institute of Mental Health, Downstate Medical Center, Univ. of Rochester
School of Medicine, Michael Reese Hospital, Univ. of Chicago, Abraham Lincoln
School of Medicine, Univ. of Illinois and Georgetown Univ. Hospital

LAB/BRANCH

Laboratory of Neuropharmacology

SECTION

None

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:

7

PROFESSIONAL:

5

OTHER:

2

SUMMARY OF WORK (200 words or less - underline keywords)

The dopaminergic agonist, bromocriptine, has been studied in 50 patients with idiopathic parkinsonism. Controlled observations indicate bromocriptine to be an active therapeutic agent, somewhat superior to others currently available. It is particularly helpful in patients experiencing certain adverse reactions to levodopa (L-dopa), such as the "on-off phenomena". Bromocriptine appears to be well tolerated, with maximum benefit achieved when combined with low doses of L-dopa. Administration of bromocriptine altered the metabolites of dopamine and serotonin in the cerebrospinal fluid (CSF). Concentrations of homovanillic acid were reduced, implying diminished presynaptic dopaminergic activity, while levels of 5-hydroxyindoleacetic acid were increased, suggesting blockade of postsynaptic serotonergic receptors. Attempts to potentiate the action of bromocriptine by inhibiting phosphodiesterase with caffeine proved unsuccessful. Bromocriptine was also administered in a controlled study to 6 patients with Huntington's chorea, following a recent report of therapeutic benefit from dopaminergic activation in this disease. However, rather than improving chorea, bromocriptine was found to induce exacerbation. This finding supports the view that choreatic movements correlate with increased activity of dopaminergic systems.

Project Description:

Clinical studies which have been completed with bromocriptine fall into 6 components:

1. Activity of dopaminergic agonist, bromocriptine, was studied in 9 patients with idiopathic parkinsonism complicated by severe "on-off" phenomena induced by L-dopa. In a "blind" self evaluating, within patient comparison, fluctuations in clinical state still occurred when L-dopa (with or without carbidopa) was replaced with bromocriptine, but were significantly reduced in frequency; patients were less parkinsonian but more dyskinetic. The observation that the "on-off" phenomena can be induced by bromocriptine complicates interpretation of these episodes in terms of pharmacokinetics of L-dopa; this may be explained by variations in receptor sensitivity or alterations in the influence of unidentified neurophysiological mechanisms which modulate striatal output.
2. A double-blind crossover study was performed in 12 patients with idiopathic parkinsonism to compare the response to bromocriptine with that of previous optimal drug treatments, including L-dopa. A 26% overall improvement occurred with bromocriptine, with rigidity, tremor and facial expression showing the greatest response. Of 8 patients taking L-dopa at the beginning of the study, 7 were taken off the drug completely. Adverse reactions were transient and dose dependent.
3. Caffeine was administered to 6 patients with idiopathic parkinsonism in an attempt to potentiate the therapeutic response to bromocriptine by inhibition of phosphodiesterase. In a double-blind study at doses of 1000 mg daily, caffeine failed to enhance the antiparkinsonian action of bromocriptine (40 mg daily) given concomitantly. Although effective in potentiating the action of L-dopa and other agonists in animal models of parkinsonism, caffeine is inactive in man.
4. Bromocriptine was administered to 20 outpatients with idiopathic parkinsonism in a 6-9 month, double-blind, randomized crossover study. Significant improvement ($p < 0.01$) occurred in overall disability scores when bromocriptine (mean daily dose 79 mg) was gradually substituted for Sinemet (14 patients) or L-dopa (6 patients); tremor, rigidity, finger dexterity, balance, gait, posture, drooling, and writing all showed significant improvement. The dose of Sinemet or L-dopa was reduced an average of 74% with the addition of bromocriptine, and 6 patients were entirely withdrawn from Sinemet. Adverse reactions were similar but somewhat more frequent than those seen with Sinemet and L-dopa.
5. Based on a recent report that another dopamine receptor agonist, apomorphine, alleviates involuntary movements of Huntington's chorea, we investigated the effects of bromocriptine in this disease. A double-blind crossover study in 6 patients showed, however, that bromocriptine induced an exacerbation. This finding supports the contention that choreatic movements correlate with hyperactivity of dopaminergic systems.

6. The effect of chronic high doses of bromocriptine and placebo was studied in 12 patients (6 parkinsonian, 6 Huntington's chorea) in a blind crossover investigation. CSF homovanillic acid (principle dopamine metabolite) levels were significantly reduced by bromocriptine; in contrast, CSF 5-hydroxy-indoleacetic acid levels were significantly increased after intravenous probenecid loading, implying increased serotonin turnover. There was no change in CSF cyclic-AMP with bromocriptine. Bromocriptine induced a significant rise of growth hormone in patients with Huntington's chorea, but not in those with parkinsonism. Prolactin levels were significantly reduced by bromocriptine in both groups of patients. In addition to its action on dopaminergic systems, bromocriptine's effect on serotonin metabolism is relevant in view of the psychiatric reactions to bromocriptine and its structural resemblance to LSD. The lack of growth hormone response to bromocriptine in patients with parkinsonism may reflect a difference in receptor sensitivity compared to patients with Huntington's chorea.

Publications:

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7. Kartzinell, R., Shoulson, I. and Calne, D.B.: Studies with bromocriptine Part II: Double-blind comparison with L-dopa in idiopathic parkinsonism. Neurology, in press.
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| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT include this space) | U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 NS 02226-01 LP |
| PERIOD COVERED September 15, 1975 to April 1, 1976 | | |
| TITLE OF PROJECT (80 characters or less) Neurochemistry of Lead Poisoning | | |
| NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT PI: Ellen K. Silbergeld Staff Fellow LP/NINCDS | | |
| COOPERATING UNITS (if any) Departments of Pediatrics and Pharmacology, Johns Hopkins Medical School, Baltimore, Maryland and John F. Kennedy Institute, Baltimore, Maryland | | |
| LAB/BRANCH Laboratory of Neuropharmacology | | |
| SECTION None | | |
| INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20014 | | |
| TOTAL MANYEARS: 0.3 | PROFESSIONAL: 0.3 | OTHER: ----- |
| SUMMARY OF WORK (200 words or less - underline keywords) Studies include <u>neurochemical</u> , <u>neuropharmacological</u> and <u>behavioral</u> investigations of rodents exposed from birth to weaning to body lead burden. The purpose of this project is to determine what neurochemical lesions are associated with lead poisoning in order to provide better therapeutic and prophylactic treatment of this disorder. Since many of the behavioral alterations and pharmacological responses in both animals and children with <u>chronic lead poisoning</u> are similar to those observed in the <u>hyperkinetic syndrome</u> these studies may also contribute significantly to the understanding of this childhood disorder. | | |

Project Description:

Objectives: (1) To study the mechanisms of lead neurotoxicity in animal models of subencephalopathic lead poisoning; (2) to investigate functioning of the central nervous system in asymptomatic children with increased lead absorption.

Methods Employed: Preclinical studies utilize rodents exposed from birth to weaning to inorganic lead via drinking water. These animals are studied for altered behavior and response to drugs. Brain tissue, blood, and urine taken from lead treated and control animals are analyzed for lead concentrations, protoporphyrin levels (blood), enzyme activity, levels of putative neurotransmitters and their metabolites, and dynamic indices of neurotransmitter function. In addition, synaptosomal and mince preparations from brain are treated in vitro with lead and synthesizing enzyme activity, transport and release functions measured.

The clinical studies, done in cooperation with J.J. Chisolm of the Lead Poisoning Clinic at the John F. Kennedy Institute and the Department of Pediatrics at Johns Hopkins, have been conducted under a research protocol approved by the Johns Hopkins Committee on Clinical Investigation. Children diagnosed as having increased body lead burden on the basis of hematologic and lead analyses are studied as inpatients for indices of altered neurotransmitter function. Quantitative urine collections taken before, during, and after chelation treatment are analyzed for levels of catecholamine metabolites. In addition, some children are studied intensively by psychometric evaluation.

Major Findings: The clinical studies on asymptomatic children have corroborated experimental results, that undue lead exposure is associated with increased urinary excretion of the catecholamine metabolites homovanillic acid (HVA) and vanillylmandelic acid (VMA). No clear correlation exists between the degree of increased HVA and any other indication of lead exposure, such as δ -amino-levulinic acid (ALA), protoporphyrin, or blood or urine lead concentration. However, it appears that there may be a significant negative correlation between HVA excretion in urine and amount of manganese excreted in urine by lead-exposed children. Further, in most cases, there is a clear response to chelation therapy; urinary HVA tends to return to normal levels as urinary ALA reaches normal range.

Studies on lead-exposed animals have mainly continued earlier work of lead on dopaminergic pathways. In vitro exposure of brain minces to lead significantly increases the release of dopamine and inhibits its reuptake. In collaboration with Dr. I. Creese, Department of Pharmacology, Johns Hopkins Medical School, studies have been initiated on the effects of in vitro lead treatment on the proposed dopamine receptor in the caudate. These studies will continue.

PUBLICATIONS:

Silbergeld, E.K. and Chisholm, J.J., Jr.: Lead poisoning: Altered urinary catecholamine metabolites as indicators of intoxication in mice and children. Science 192:153-155, 1976.

Silbergeld, E.K.: Neurochemical and pharmacological studies of central nervous system lead toxicology. In Carnow, B.W. (Ed.): Health Effects of Occupational Lead and Arsenic Exposure. USDHEW, CDC-NIOSH, 1976, pp. 74-85.

- . Silbergeld, E.K.: Neuropharmacology of hyperkinesis. In Easman, W. and Valzelli, L. (Eds.): Current Developments of Psychopharmacology. Vol. 3, in press.
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| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (On NOT use this space) | U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 NS 02227-01 LP | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| PERIOD COVERED July 1, 1975 to June 30, 1976 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| TITLE OF PROJECT (80 characters or less) Pharmacological manipulations of Neurotransmitter Metabolism in the Waking Primate | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| <table style="width: 100%; border: none;"> <tr> <td style="width: 25%; vertical-align: top;">Principal Investigators:</td> <td style="width: 45%;">Mark Perlow</td> <td style="width: 20%;">Staff Fellow</td> <td style="width: 10%;">LP/NINCDS</td> </tr> <tr> <td></td> <td>Thomas N. Chase</td> <td>Chief</td> <td>LP/NINCDS</td> </tr> <tr> <td style="vertical-align: top;">Other Investigators:</td> <td>Michael Ebert</td> <td>A/Chief, SET</td> <td>LCS/NIMH</td> </tr> <tr> <td></td> <td>Irwin Kopin</td> <td>Chief</td> <td>LCS/NIMH</td> </tr> <tr> <td></td> <td>Edna Gordon</td> <td>Chemist</td> <td>LCS/NIMH</td> </tr> <tr> <td></td> <td>R.C. Lake</td> <td>Res. Assoc.</td> <td>LCS/NIMH</td> </tr> <tr> <td></td> <td>Michael Ziegler</td> <td>Pharmacologist</td> <td>LCS/NIMH</td> </tr> <tr> <td></td> <td>Sanford Markey</td> <td>Pharmacologist</td> <td>LCS/NIMH</td> </tr> <tr> <td></td> <td>Barry Pestoff</td> <td>Med. Off.</td> <td>MN/NINCDS</td> </tr> <tr> <td></td> <td>Frederic C. Bartter</td> <td>Chief</td> <td>HE/NHLI</td> </tr> <tr> <td></td> <td>Howard Hoffman</td> <td>Math Stat</td> <td>OAEB/NICHD</td> </tr> <tr> <td></td> <td>Ronald Kartzinell</td> <td>Clinical Associate</td> <td>LP/NINCDS</td> </tr> <tr> <td></td> <td>Donald Calne</td> <td>Clinical Director</td> <td>OD/NINCDS</td> </tr> <tr> <td></td> <td>Charles Dinarello</td> <td>Guest Worker</td> <td>LCI/NIAID</td> </tr> <tr> <td></td> <td>Sheldon Wolff</td> <td>Chief</td> <td>LCI/NIAID</td> </tr> </table> | | | Principal Investigators: | Mark Perlow | Staff Fellow | LP/NINCDS | | Thomas N. Chase | Chief | LP/NINCDS | Other Investigators: | Michael Ebert | A/Chief, SET | LCS/NIMH | | Irwin Kopin | Chief | LCS/NIMH | | Edna Gordon | Chemist | LCS/NIMH | | R.C. Lake | Res. Assoc. | LCS/NIMH | | Michael Ziegler | Pharmacologist | LCS/NIMH | | Sanford Markey | Pharmacologist | LCS/NIMH | | Barry Pestoff | Med. Off. | MN/NINCDS | | Frederic C. Bartter | Chief | HE/NHLI | | Howard Hoffman | Math Stat | OAEB/NICHD | | Ronald Kartzinell | Clinical Associate | LP/NINCDS | | Donald Calne | Clinical Director | OD/NINCDS | | Charles Dinarello | Guest Worker | LCI/NIAID | | Sheldon Wolff | Chief | LCI/NIAID |
| Principal Investigators: | Mark Perlow | Staff Fellow | LP/NINCDS | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| | Thomas N. Chase | Chief | LP/NINCDS | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Other Investigators: | Michael Ebert | A/Chief, SET | LCS/NIMH | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| | Irwin Kopin | Chief | LCS/NIMH | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| | Edna Gordon | Chemist | LCS/NIMH | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| | R.C. Lake | Res. Assoc. | LCS/NIMH | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| | Michael Ziegler | Pharmacologist | LCS/NIMH | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| | Sanford Markey | Pharmacologist | LCS/NIMH | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| | Barry Pestoff | Med. Off. | MN/NINCDS | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| | Frederic C. Bartter | Chief | HE/NHLI | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| | Howard Hoffman | Math Stat | OAEB/NICHD | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| | Ronald Kartzinell | Clinical Associate | LP/NINCDS | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| | Donald Calne | Clinical Director | OD/NINCDS | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| | Charles Dinarello | Guest Worker | LCI/NIAID | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| | Sheldon Wolff | Chief | LCI/NIAID | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| COOPERATING UNITS (if any) National Institute of Mental Health, National Heart and Lung Institute, National Institute of Child Health and Human Development and National Institute of Allergy and Infectious Diseases | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| LAB/BRANCH Laboratory of Neuropharmacology | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| SECTION | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20014 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| <table style="width: 100%; border: none;"> <tr> <td style="width: 33%;">TOTAL MANYEARS:</td> <td style="width: 33%;">PROFESSIONAL:</td> <td style="width: 33%;">OTHER:</td> </tr> <tr> <td style="text-align: center;">1.0</td> <td style="text-align: center;">1.0</td> <td style="text-align: center;">0.0</td> </tr> </table> | | | TOTAL MANYEARS: | PROFESSIONAL: | OTHER: | 1.0 | 1.0 | 0.0 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| TOTAL MANYEARS: | PROFESSIONAL: | OTHER: | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| 1.0 | 1.0 | 0.0 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| SUMMARY OF WORK (200 words or less - underline keywords) These investigations are designed to substantiate and extend <u>in vitro neurochemical</u> observations to the integrated waking <u>primate</u> and provide a <u>primate</u> system in which it is possible to devise investigative strategies or paradigms for investigations of neurochemical and neuropharmacological aspects of normal and disordered central nervous system function in man. Particular emphasis has initially been placed on studies of the <u>circadian</u> fluctuations of central <u>monoamines</u> and the test of newly devised labelling procedures, using <u>non-radioactive isotopes</u> , for clinical investigations of <u>neurotransmitter</u> metabolism. | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |

Project Description:

Using the recently assembled primate facility, the objectives of this project are to extend in vitro neurochemical observations to the integrated waking primate and to provide a primate system in which it is possible to devise investigative strategies or paradigms that can be applied to investigations into neurochemical and neuropharmacological aspects of central nervous system function in man.

Using a recently assembled primate facility, we have performed experiments on the chronically chair-restrained waking rhesus monkey adapted to pre-set environmental and dietary conditions. Cerebrospinal fluid is obtained continuously on a 24 hour basis from the lateral ventricle. By this means it was found that the central turnover of dopamine, norepinephrine and serotonin, as estimated by levels of their principle metabolites, exhibited a characteristic circadian rhythmicity. Variations in these neurotransmitter amines over the 24-hour light-dark cycle were generally coincident with changes in CSF concentrations of cyclic AMP as well as with body activity and brain temperature. Using similar procedures circadian fluctuations in the CSF content of sodium and potassium were also found which differ from those reported to occur in the urine of man. Future studies will use drugs to further explore the relationship between levels of neurotransmitters or their metabolites in ventricular CSF, the activity of specific central neurohumoral systems, and various behavioral parameters.

In collaboration with NIMH investigators studies have been initiated on the use of stable (non-radioactive) isotopes for the evaluation of central neurotransmitter metabolism. Preliminary results indicate that O_{18} (given in a breathing mixture containing 95% O_{18}) and deuterated methionine (given intravenously) provide sufficient labelling of catecholamine metabolites in ventricular CSF of subhuman primates to allow reliable turnover estimates.

These collaborative studies will continue with special emphasis being placed on the development and validation of techniques which are directly applicable to man.

Publications:

None

| | | | | | |
|---|--|---|--|--|--|
| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | | U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | | PROJECT NUMBER Z01 NS 02228-01 LP | |
| PERIOD COVERED December 1, 1975 to June 30, 1976 | | | | | |
| TITLE OF PROJECT (80 characters or less) Pathogenesis of Cerebral Ischemia in Animal Stroke Models | | | | | |
| NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT | | | | | |
| P.I. William E. Lightfoote, II | | Medical Officer | | LP:NINCDS | |
| Thomas N. Chase | | Chief | | LP:NINCDS | |
| Other: John L. Sever | | Chief | | ID:NINCDS | |
| Ayub K. Ommaya | | Acting Chief | | SN:NINCDS | |
| Edward A. Cudahy | | Associate | | CDP:NINCDS | |
| Gaetano F. Molinari | | Chairman, Neurology Dept. George Washington Univ. Medical Center | | Washington, DC | |
| COOPERATING UNITS (if any) Surgical Neurology Branch, NINCDS, NIH, Infectious Diseases Branch, NINCDS, NIH, Communicative Disorders Program, NINCDS, NIH, George Washington University, Dept. of Neurology, Washington, DC. | | | | | |
| LAB/BRANCH Laboratory of Neuropharmacology | | | | | |
| SECTION Stroke Models Unit | | | | | |
| INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, MD 20014 | | | | | |
| TOTAL MANYEARS: 1.5 | | PROFESSIONAL: 1.0 | | OTHER: 0.5 | |
| SUMMARY OF WORK (200 words or less - underline keywords) | | | | | |
| <p>The objective of the Stroke Models Unit is the study of experimental <u>cerebro-vascular disease</u>. Physiologic, biochemical, and histopathologic markers are monitored in a variety of different animal species interfaced with pre-, intra-, and post-ictal therapies for <u>cerebral ischemic events</u>. The latter investigations should provide data relevant to the clinical management of cerebrovascular disease.</p> | | | | | |

Sampling and on-line processing of neurophysiologic parameters have been extended to primate stroke models to identify changes following middle cerebral artery occlusion more rapidly than is possible through visual inspection of electroencephalographic records. Four channels of bipolar electroencephalogram were monitored from Rhesus monkeys undergoing segmental middle cerebral artery occlusion using a silicone-rubber cylindrical embolus. Electroencephalograms were recorded on digital cassette tape and their power spectra displayed using the Fast Fourier Transform. Preliminary results indicate that changes in cortical rhythm occur sooner than previously suspected on the basis of simple visual inspection of electroencephalographic records.

IV. Barbiturate Protection

Barbiturates are reputed to protect the cerebral cortex during an ischemic episode presumably by lowering CMR-O₂ and CMR-glu. In order to test this hypothesis in the Mongolian gerbil stroke model, 130 animals underwent unilateral carotid artery ligation; of these 66 were given a non-barbiturate anesthetic (cyclohexylamine), while another 64 animals received a barbiturate. The yield of infarcted hemispheres was 82% in the cyclohexylamine treated group and 50% in barbiturate treated animals. These latter findings are consonant with those of other investigators and with the known anatomical variance of 40% of adult gerbils who lack significant vascular communication between the vertebrobasilar and carotid arterial circulations. Furthermore, the data support the hypothesis that hypometabolic therapy slows the momentum of the ischemic insult and therefore may be useful in the treatment of stroke victims.

Publications: None

ANNUAL REPORT

July 1, 1975 through June 30, 1976
Infectious Diseases Branch, IRP
National Institute of Neurological and
Communicative Disorders and Stroke

I. Responsibility of the Branch

The responsibility of the Infectious Diseases Branch is to carry out planned directed research programs concerned with infections which damage the human nervous system. The Branch is divided into five sections: 1) Immunochemistry and Clinical Investigations, 2) Experimental Pathology, 3) Neurogenetics, 4) Neurovirology and 5) Electron Microscopy. These sections utilize the techniques of immunology, clinical investigations, including human volunteers and clinical trials; experimental pathology with non-human primates, virology, bacteriology, mycoplasmaeology, genetics, neurovirology, human tissue culture and electron microscopy.

II. Program Segments

The program segments are: a) Perinatal, b) Acute and c) Chronic. In each segment we are concerned with 1) etiology and diagnosis, 2) treatment, and 3) prevention.

The research areas in the program segments include:

1. Perinatal

Develop and utilize large scale methods to study the relation between viral, bacterial, mycoplasma and protozoa infections in the perinatal period and birth defects, related abnormalities and pediatric neurological diseases. Investigate approaches to early diagnosis, treatment and prevention using combined laboratory and clinical studies.

2. Acute

Investigate agents which may be responsible for acute neurological diseases such as meningitis, encephalitis, Bells' Palsy, and tic douloureux as well as possible methods for rapid diagnosis, treatment and prevention.

3. Chronic

Study chronic neurological diseases such as multiple sclerosis, amyotrophic lateral sclerosis, Parkinson's disease, subacute sclerosing panencephalitis, Alzheimer's and Pick's disease and epilepsy using combined tissue culture, immunological serological, genetic electron microscopic and clinical approaches for possible infectious etiologies. Whenever possible, explore methods for early diagnosis, treatment and prevention.

III. Section Activities

1. Section on Immunochemistry and Clinical Investigations

A. Perinatal

The Section is responsible for the research and the analysis of Collaborative Perinatal Project sera and data for infection in 60,000 pregnancies. The approaches being used include: 1) Clinical infections - correlation with pregnancy outcomes, 2) Serological investigation of 6000 abnormals and 6000 controls, and 3) High IgM among 30,000 children as a method to identify infected children.

Additional studies include high risk children and infections in relation to infertility and abortions. A study is being conducted on virus C-particles in placentas and fetal tissues. Vaccines for herpes, cytomegaloviruses and toxoplasmosis are under development.

B. Acute

New limulus tests and counter immunoelectrophoresis are being studied for the rapid diagnosis of meningitis. These tests along with LDH enzyme determinations appear very useful. Human volunteer investigations of rubeola and rubella vaccines are being conducted at the Petersburg Federal Reformatory. Defective delinquents are being tested for herpes antibodies.

C. Chronic

Clinical studies of the treatment of SSPE with "levamisole" are being initiated jointly at the University of Tennessee. Special genetic (HLA) and serological investigations of MS and Parkinson's disease patients are in progress.

New radioimmune and enzyme linked methods for antibody assays are under development.

2. Section on Experimental Pathology

A. Perinatal

This section is conducting extensive studies of experimental monkeys which develop CNS and other damage when infected in utero. Current agents include flu, Reo, VEE and mumps viruses. The value of new chemotherapeutic materials is under investigation. The effect of Strep B meningitis is being investigated in detail.

B. Acute

Transmission studies of Toxoplasmosis, herpes II and varicella are under investigation. Most of the agents can result in chronic infections. Treatment studies for CNS herpes are in progress.

C. Chronic

Intrauterine inoculation of pregnant monkeys with MS and other tissues is being continued by the Section. Chronic infection with SHF virus is being studied in detail. The role of herpes in cancer is being studied in collaboration with the National Cancer Institute.

3. Section on Neurogenetics

This section is responsible for combined genetic-infection studies of neurological diseases. These current studies include HLA-MLC studies of patients with MS in families and twins as well as patients with ALS and Reye's syndrome.

The section is also investigating Tourette syndrome, dystonia, Huntington's chorea and families with progressive myoclonic disease. CNS neoplasia are being studied for families with Central Neurofibromatosis and Von Hippel-Lindau syndrome.

4. Section on Neurovirology

A. Perinatal

This section is conducting studies of the frequency of perinatal, amniotic, ovarian, and cervical infections. In addition, a study of neonatal deaths associated with infections is being conducted.

B. Acute

Chemotherapy of Flu, Reo and other agents are under investigation with the Section on Experimental Pathology.

C. Chronic

Mechanisms of cellular immunity to various viruses are being tested using patient material from individuals with MS, SSPE, and other neurological diseases.

The pathogenesis of PML (SV₄₀) like agents are under study with the Section on Experimental Pathology. Also possible virus etiologies of MS are under investigation using a variety of techniques.

The immune response of monkeys to herpes virus is being studied. New antiviral drugs are being tested in vitro and in experimental animals with chronic infection.

Attempts to isolate a virus related to MS are being conducted in mice and tissue culture. A new ELISA enzyme test for antibody to infectious agents is under development. Biochemical tests for suppressed viruses are being used in studies of SSPE-Measles and MS.

5. Section on Electron Microscopy

This new section is using immuno electron microscopy in studies of MS, SSPE, visna, and scrapie. New freeze fracture methods are being employed. The mechanisms of chronic infection in CNS tissue are being studied and the interaction between virusea and lymphocytes is being investigated.

IV. Findings

1. Perinatal

A. Cytotoxic Antibodies Increased in CNS and Heart Defects

Current studies indicate some increase in cytotoxic antibodies in sera from pregnant women who delivered children with various CNS and heart defects. More detailed studies are in progress.

B. Infection and Low Birth Weight in an Industrialized Society

Women with urinary tract infections during pregnancy had increased rates of low birght weight children, abortions, stillbirths and neonatal deaths. These associations held when controlled for socio-economic level and other variables. All women were treated for these symptomatic infections. These studies indicate that common urinary infections are important causes of poor fetal development and death.

C. Hepatitis A not Causally Associated with Down's Syndrome

Women who delivered children with Down's syndrome have been reported to have increased rates of Hepatitis A infections. No such association with Hepatitis A was found in these studies. Thus, Hepatitis A was not found to be etiologically important for Down's syndrome in these cases.

D. Brain and Eye Malformations Induced in Fetal Rhesus Monkeys by Venezuelan Equine Encephalitis Virus

Our investigations have shown that vaccine VEE virus produces porencephalic cysts and bilateral cataracts in monkeys infected in utero. These findings are in agreement with clinical reports from Central and South America. This study has brought recognition to the teratogenicity of this virus

2. Acute

A. Immune Serum Globulin (Human) Reduces Mortality of Newly Imported Rhesus Monkeys

The use of outdated human ISG significantly reduced the mortality of imported rhesus monkeys. These observations were particularly valuable since restriction on importations of monkeys make these animals particularly valuable. The protection seems greatest for common respiratory and gastrointestinal diseases. Studies in low birthweight children are being considered.

B. Viral Diarrhea in Monkeys Due to the Human Reo Like Agent

Studies in rhesus monkeys have demonstrated susceptibility of colostrum deprived newborns to the newly recognized human Reo like virus. The findings now demonstrate the pathogenicity of this agent.

C. Transmission and Prevention of Simian Hemorrhagic Fever in Rhesus Monkeys

Infection with SHF virus can completely destroy a rhesus monkey colony in less than two weeks. Following the loss of over 200 rhesus monkeys with this disease, Dr. London initiated studies on the same and spread of this disease as well as possible methods for prevention. Through these studies he demonstrated: 1) The Patas monkey is a constant asymptomatic carrier of SHF 2) Transmission from patas to rhesus results in immediate spread of the disease and death of rhesus animals in a few days 3) Isolation of Patas will prevent transmission 4) Poly Ic Poly D given to exposed rhesus will protect them from disease. These findings are of considerable importance for all primate centers.

3. Chronic

A. Subacute Sclerosing Panencephalitis Increased in Individuals Who are HLA Type W29

A survey of patients with SSPE in the United States showed a very significant association with the genetically determined HLA type W29. This study indicated a genetic component may be important along with the measles virus in patients with SSPE.

B. Subacute Sclerosing Panencephalitis: Destruction of Brain Cells by Antibody and Complement

Tests of brain cells from patients with SSPE showed that SSPE sera together with complement caused destruction of the brain cells. These findings suggest the method of destruction of brain tissue in patients with this disease.

C. Specific Inhibition of Cellular Immunity in SSPE

Specific inhibitions of measles cellular immunity were found in the sera and CSF of patients with SSPE. The CSF levels were approximately ten times as great as the serum levels. These findings suggest that "blocking factor" may be a major factor in the pathogenesis of SSPE.

D. Defective Bud Formation in Chronically Infected Cells with SSPE Virus

Electron microscopic studies of chronically infected tissues with SSPE measles virus have shown defective virus budding. These studies show a specific defect of virus maturation with this chronic virus infection.

CONTRACT NARRATIVE
Infectious Diseases Branch, IRP, NINCDS
Fiscal Year 1976

THE UNIVERSITY OF WISCONSIN (N01-NS-4-2308)

Title: Study of the Use of Zonal Ultracentrifugation and Affinity Chromatography in the Etiology and Diagnosis of Multiple Sclerosis.

Contractor's Project Director: Dr. Steven E. Kornguth

Current Annual Level: \$113,888.00

Objectives: The basic objective of this contract is to investigate the possible etiology and diagnosis of MS. To gain information into these areas we desired to utilize the techniaue of zonal ultracentrifugation to purify and concentrate subcellular elements in MS brain tissue and utilize electronmicroscopy, affinity chromatography and biochemistry to identify antigens and/or antibody against subcellular particles. Hopefully, we will gain some insight into the pathogenic process and identity of possible etiologic agents.

Major Findings: Studies on a brain obtained shortly after death was subjected to differential centrifugation and CSCI density gradient concentration. Two crystalline components and rigid tubules were observed by EM. They range in size 160-220 A and 320-380 A for the crystalline structures and rigid tubules 320-450A.

Significance to the NINCDS Program and Biomedical Research: The goal of the NINCDS is to carry out planned directed research programs concerned with the diseases which damage the human nervous system. Multiple Sclerosis affects over 200,000 in the United States and many more throughout the World. Recent evidence suggests a possible infectious etiology for MS and these findings need to be investigated. The exceptional resolving capabilities of rate zonal ultracentrifugation and the use of affinity chromatography should help to identify possible etiologic agents.

Proposed Course of the Project: Samples from the rate zonal separation of additional MS brain tissue will be passed through affinity chromatography columns labeled with the gamma globulin of MS spinal fluid. Fractions will then be collected from these columns, materials pelleted and observed by electronmicroscopy and biochemical techniques.

CONTRACT NARRATIVE
Infectious Diseases Branch, IRP, NINCDS
Fiscal Year 1976

SCRIPPS CLINIC AND RESEARCH FOUNDATION (N01-NS-4-2309)

Title: Detection of Viral Genomes in Human Neurological Diseases

Contractor's Project Director: Dr. William J. Meinke

Current Annual Level: \$63,387.00

Objectives: Contractor will attempt to demonstrate the presence or absence of viral genes in cells derived from patients with chronic neurological diseases, principally multiple sclerosis, amyotrophic lateral sclerosis, and Alzheimer's disease. The experimental approach is to analyze cellular nucleic acids derived from Central Nervous System tissues by extremely sensitive molecular DNA-DNA and DNA-RNA hybridization techniques.

Major Findings: Viral probes for herpes virus type 1 and type 2 have been prepared and are presently being used on extracted material. Results have been negative for these probes when tested against 3 MS and 3 ALS brain extracted materials. A study of seven human brain tumors showed one, a glioblastoma, to contain DNA base sequences homologous to SV₄₀ DNA.

Significance to the NINCDS Program and Biomedical Research: Chronic neurological diseases may be caused by the incorporation of viral nucleic acids into brain cells and thereby control the production of cell surface changes without the expression of complete virus. Co-cultivation techniques and other methods of isolating virus may not be possible. With the use of radioactive probes high sensitivities for the detection of viral nucleic acids is now possible.

Proposed Course of the Project: Additional DNA viral probes will be prepared using the "nick-repair" enzymatic reaction. DNA will be extracted from other neurological diseases and the experimental approach as previously described will be utilized. Reliable methods for the purification of brain RNA and for isolating brain messenger RNA have been developed. Synthesizing complementary DNA from viral RNA and used as probes on tissue is now being developed.

CONTRACT NARRATIVE
Infectious Diseases Branch, IRP, NINCDS
Fiscal Year 1976

DEPARTMENT OF PEDIATRICS, UNIVERSITY OF TENNESSEE (PH43-68-17)

Title: Identification of Specific Antibodies in the Serum IgM Fraction of High Risk Infants

Contractor's Project Director: Dr. Sheldon Korones, M.D.

Current Annual Level: \$36,796

Objective: The study is designed to assess the validity and clinical usefulness of specific tests for serum IgM for the identification of antibodies to rubella, cytomegalovirus, toxoplasmosis, herpesvirus, and syphilis. When these diseases are identified as being present in the children, appropriate therapeutic approaches are used. Recent studies have included the investigation of group B streptococcal infections.

Major Findings: Approximately 3,000 infants have been tested to date. Serial blood specimens are collected twice each week from each study infant for the duration of the nursery stay. Venous blood obtained from infants with IgM concentration is in excess of 20 mgm percent in the first two weeks of life and 30 mgm percent between the third and fourth weeks. Virus cultures are obtained from all infants with high IgM levels. Clinical evaluation data is being maintained for correlation with the serologic findings.

Significance to the NINCDS Program and Biomedical Research: The detection of high risk infants with congenital infections is hampered by the lack of specific tests to identify these children. Recent studies have indicated that elevated IgM levels are present in at least 80 percent of children with congenital infection. The present studies will provide direct information as to the usefulness of specific IgM antibody determinations for five major infectious agents as a means of detecting not only the possible presence of congenital infection but the specific infection which requires treatment. New methods for the removal of IgG will be added to the IgM, FA procedures.

Proposed Course of the Project: The project is completing its third year. Specific FA tests are now being finished for syphilis, toxoplasmosis and cytomegalovirus using new kits now available. The high rate of Group B meningitis and fatal newborn streptococcal disease will be considered in a new proposal.

CONTRACT NARRATIVE
Infectious Diseases Branch, IRP, NINCDS
Fiscal Year 1976

THE JOSEPH STOKES, JR. RESEARCH INSTITUTE OF THE CHILDREN'S HOSPITAL OF
PHILADELPHIA (N01-NS-5-2311)

Title: Immune Electron Microscopic Techniques as an Aid for Determination of the Etiology of Multiple Sclerosis.

Contractor's Project Director: Dr. Klaus Hummeler

Current Annual Level: \$65,630.00

Objectives: The objective of this contract is to investigate the use of the Immune Electron Microscopic (IEM) technique as an aid in determining the etiology of MS. Initially the sensitivity of the test will be determined. Serum and spinal fluid from MS patients will be reacted with brain homogenate from MS and non-MS patients.

Major Findings: Efforts to identify a viral agent as the cause of MS has been initiated. Completion of pilot studies utilizing polio and herpes viruses indicate that the IEM technique is sensitive enough to detect viral antigens in brain homogenate and cerebral fluids (CFS). Preliminary studies have been initiated using human MS serum or CSF as antibody and human MS or control brain homogenates as antigen to look for the presence of antibody-viral complexes.

Significance to the NINCDS Program and Biomedical Research: The mission of the NINCDS is to carry out research programs concerned with diseases affecting the central nervous system. Recent evidence suggest a possible infectious etiology for MS. The use of the IEM technique has proven highly effective in identification of several diseases whose etiology had been obscure. Use of the IEM technique in MS studies may help identify possible etiological agents missed by more conventional techniques.

Proposed Course of the Project: Examination of brain homogenates from MS patients and controls using serum and CSF from MS patients and controls will be continued. A supplement has been proposed to be added to this contract. With additional funding the IEM technique will be used as an aid to identify the Multiple Sclerosis Associated Agent (MSAA). These studies will include efforts to identify and characterize the agent in human brain material, mouse brain material and tissue culture cells.

CONTRACT NARRATIVE
Infectious Diseases Branch, IRP, NINCDS
Fiscal Year 1976

THE MOUNT SINAI SCHOOL OF MEDICINE (N01-NS-5-2309)

Title: Virological Studies in Parkinson's Disease

Contractor's Project Director: Dr. Teresita Elizan

Current Annual Level: \$95,548

Objectives: Contractor will attempt to demonstrate the presence or absence of viruses, viral genes in cells derived from patient brains with Parkinson's disease. The experimental approach is to grow tissue cell cultures from brains and determine the presence of viruses by Electron Microscopy co-cultivation, and various antibody techniques. Also brain tissues will be analyzed by deriving the cellular nucleic acids and determining the possible presence of viral DNA or RNA through sensitive hybridization techniques.

Major Findings: A total of 13 idiopathic Parkinson's and one post-encephalitic Parkinson's and four matched control brains have been obtained. Six brains have been successfully cultured. Various areas of the brains were grown in these studies. They are now being studied with the methods previously described. One of the brains have been checked by hybridization for influenza A and herpes simplex I. No significant hybridization could be found with the herpes CNA probe. Influenza results are not yet complete. A total of 171 serum samples and 136 cerebrospinal fluid samples have been obtained. These include Parkinson patients and matched normal controls. These will be tested in a serological screen at NIH.

Significance to the NINCDS Program and Biomedical Research: Parkinson's Disease may be caused by a viral infection. Co-cultivation techniques and other methods of isolating incomplete virus will be performed to determine the presence of virus. The use of radioactive probes with high sensitivities for the detection of viral nucleic acids now makes this approach possible.

Proposed Course of the Project: Additional brains will be grown and studied for the presence of virus. Additional DNA and RNA probes will be prepared and further hybridization studies done. Serological testing of the sera and CSF samples will be done against a number of different viral antigens to determine if there is any significant titer to a particular virus.

CONTRACT NARRATIVE
Infectious Diseases Branch, IRP, NINCDS
Fiscal Year 1976

UNIVERSITY OF FLORIDA (N01-NS-5-2318)

TITLE: The Viral Induction of Malformations in Developing Rhesus Monkeys.

Contractor's Project Director: Dr. Alvin F. Moreland

Current Annual Level: \$167,936.00

Objectives: To determine if Influenza A, Mumps and Western Equine Encephalitis (WEE) viruses can be transmitted from the blood of the pregnant Rhesus monkey to the fetus. Should these viruses not cross the placenta of the monkey, then intra-amniotic inoculations will be made.

Inoculations of these viruses will be given at various times of gestation: 25, 50, 80 days.

Major Findings: There have been delays in obtaining monkeys for this study as well as isolation equipment needed to contain the animals during acute infection of the virus. All of these are now accomplished and the monkeys are either in quarantine or are under observation to establish bleeding cycles so they can be time mated. To date, there are 12 animals in early pregnancy. No inoculations have been made.

Significance to the NINCDS Program and Biomedical Research: The goal of the NINCDS is to carry out planned directed research programs concerned with the diseases which damage the human nervous system. Studies done in the Infectious Diseases Branch of NINCDS have established that the three human viral agents to be investigated under this contract are teratogenic when inoculated into non-human primate fetuses. The anomalies are mainly of the central nervous system. The work planned under this contract should establish if these viral agents are teratogens when given to the pregnant, non-human primate.

Proposed Course of the Project: The animals will be inoculated and processed as outlined in the work scope of the contract.

CONTRACT NARRATIVE
Infectious Diseases Branch, IRP, NINCDS
Fiscal Year 1976

MELOY LABORATORIES, SPRINGFIELD, VIRGINIA (N01-NS-2-2306)

Title: Herpes Virus Induction of Cervical Cancer in Cebus Monkeys

Contractor's Project Director: Dr. D. Lewis Sly

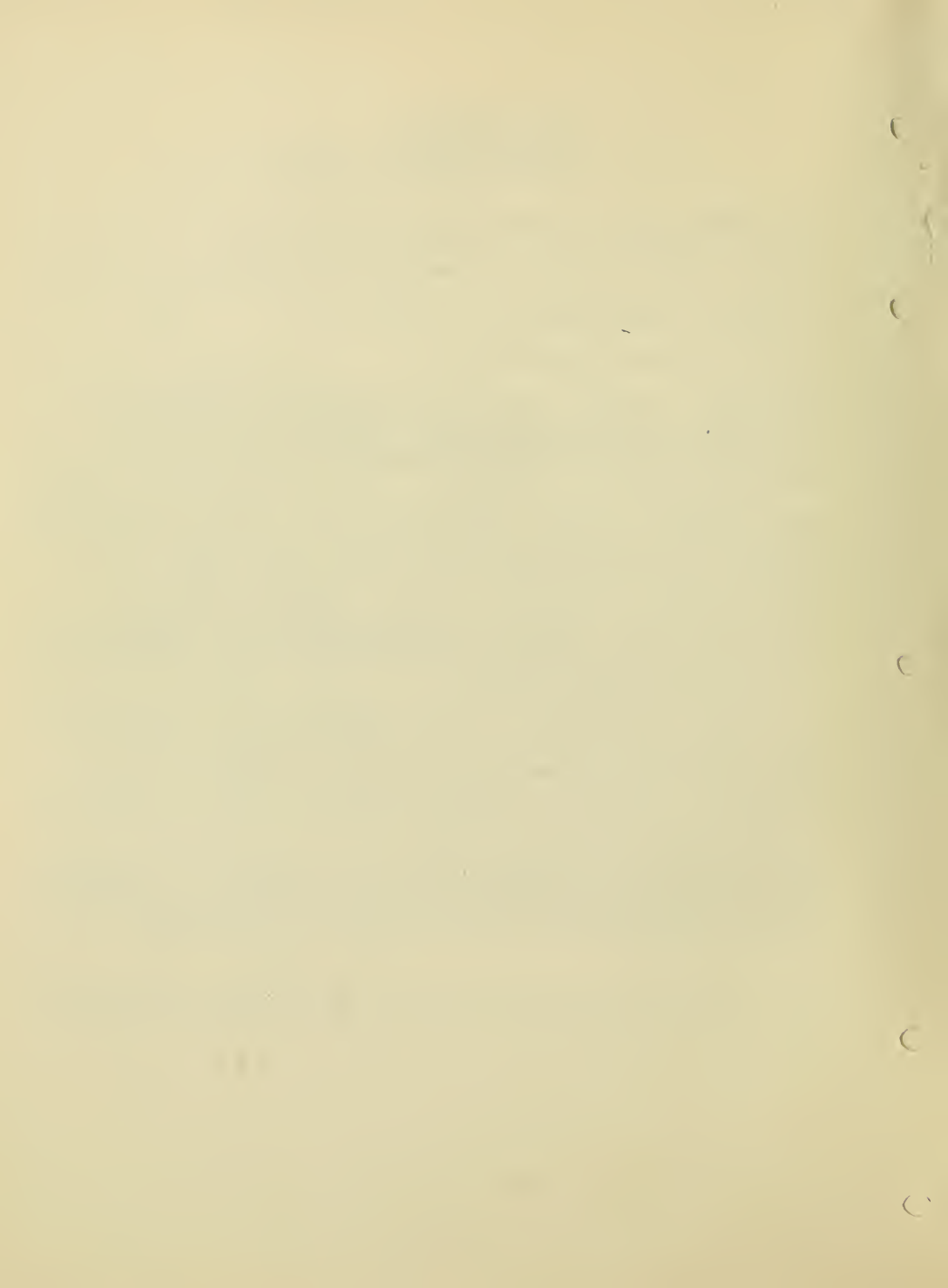
Current Annual Level: \$270,976.00

Objectives: An attempt to show a relationship between infection with Herpes Simplex Virus, type 2 and cervical cancer in an animal model (cebus spp.). By constant monitoring of the animals, it is possible to follow the course of infection and developing anaplasia, if it develops.

Major Findings: Persistent reinoculation of cebus monkeys with HSV-2 eventually results in the infection of all inoculated animals. Detectable neutralizing serum antibodies persist beyond six months and reinfection after reinoculation occurs at a low rate. Spontaneous recurrent infections occur in the females and venereal transmission to males has been demonstrated. The virus has been demonstrated from sacral ganglia of infected females by explant-cocultivation techniques. Persistent mild cytological anaplasia was detected in a small number of virus infected, but no control animals.

Significance to the NINCDS Program and Biomedical Research: The role of HSV-2 infection in perinatal disease, cervical carcinoma and chronic neurological disease in humans stimulated the development of a non-human primate model to study the pathogenic and oncogenic potential of this virus.

Proposed Course of the Project: Reinoculation of the animals with virus or control material will continue at nine month intervals. The serological behavior against viral and nonviral antigens will be studied. Spontaneous recurrent infection and colposcopic evaluations will be performed.



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|---|---|--|--------------------------|--|-------------------------|-------------------------|-----------------------|--|---------------------|--|----------------|---|------------------|----------------------------------|------------------|----------------------------------|--|----------------------|-------------------------|--|--------------------|-------------------------------|
| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 NS 00402-20 ID | | | | | | | | | | | | | | | | | | | | |
| PERIOD COVERED July 1, 1975 through June 30, 1976 | | | | | | | | | | | | | | | | | | | | | | |
| TITLE OF PROJECT (80 characters or less) Perinatal Infections Causing Damage to the Child - Collaborative Perinatal Project | | | | | | | | | | | | | | | | | | | | | | |
| NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT <table style="width: 100%; border: none;"> <tr> <td style="width: 15%;">PI:</td> <td style="width: 40%;">Dr. John L. Sever</td> <td style="width: 45%;">Chief, IDB, IRP, NINCDS</td> </tr> <tr> <td rowspan="5">Others:</td> <td>Dr. David Fuccillo</td> <td>Head, Section on Neurovirology, IDB IRP, NINCDS</td> </tr> <tr> <td>Dr. Jonas Ellenberg</td> <td>Asst. Head, Section on Mathematical Statistics, OBE, OD, NINCDS</td> </tr> <tr> <td>Mrs. Anita Ley</td> <td>Head, Unit on Immunochemistry, IDB IRP, NINCDS</td> </tr> <tr> <td>Mrs. Renee Traub</td> <td>Microbiologist, IDB, IRP, NINCDS</td> </tr> <tr> <td>Mrs. Flora Moder</td> <td>Microbiologist, IDB, IRP, NINCDS</td> </tr> <tr> <td></td> <td>Mrs. Dorothy Edmonds</td> <td>Nurse, IDB, IRP, NINCDS</td> </tr> <tr> <td></td> <td>Mrs. Eudora Beadle</td> <td>Statistician, OBE, OD, NINCDS</td> </tr> </table> | | | PI: | Dr. John L. Sever | Chief, IDB, IRP, NINCDS | Others: | Dr. David Fuccillo | Head, Section on Neurovirology, IDB IRP, NINCDS | Dr. Jonas Ellenberg | Asst. Head, Section on Mathematical Statistics, OBE, OD, NINCDS | Mrs. Anita Ley | Head, Unit on Immunochemistry, IDB IRP, NINCDS | Mrs. Renee Traub | Microbiologist, IDB, IRP, NINCDS | Mrs. Flora Moder | Microbiologist, IDB, IRP, NINCDS | | Mrs. Dorothy Edmonds | Nurse, IDB, IRP, NINCDS | | Mrs. Eudora Beadle | Statistician, OBE, OD, NINCDS |
| PI: | Dr. John L. Sever | Chief, IDB, IRP, NINCDS | | | | | | | | | | | | | | | | | | | | |
| Others: | Dr. David Fuccillo | Head, Section on Neurovirology, IDB IRP, NINCDS | | | | | | | | | | | | | | | | | | | | |
| | Dr. Jonas Ellenberg | Asst. Head, Section on Mathematical Statistics, OBE, OD, NINCDS | | | | | | | | | | | | | | | | | | | | |
| | Mrs. Anita Ley | Head, Unit on Immunochemistry, IDB IRP, NINCDS | | | | | | | | | | | | | | | | | | | | |
| | Mrs. Renee Traub | Microbiologist, IDB, IRP, NINCDS | | | | | | | | | | | | | | | | | | | | |
| | Mrs. Flora Moder | Microbiologist, IDB, IRP, NINCDS | | | | | | | | | | | | | | | | | | | | |
| | Mrs. Dorothy Edmonds | Nurse, IDB, IRP, NINCDS | | | | | | | | | | | | | | | | | | | | |
| | Mrs. Eudora Beadle | Statistician, OBE, OD, NINCDS | | | | | | | | | | | | | | | | | | | | |
| COOPERATING UNITS (if any) <table style="width: 100%; border: none;"> <tr> <td style="width: 33%;">Johns Hopkins University</td> <td style="width: 33%;">University of California, Los Angeles and Kaiser</td> <td style="width: 33%;"></td> </tr> <tr> <td>University of Tennessee</td> <td>Hospital, Los Angeles</td> <td></td> </tr> <tr> <td></td> <td colspan="2">Collaborating Inst. in the Collaborative Perinatal Project.</td> </tr> </table> | | | Johns Hopkins University | University of California, Los Angeles and Kaiser | | University of Tennessee | Hospital, Los Angeles | | | Collaborating Inst. in the Collaborative Perinatal Project. | | | | | | | | | | | | |
| Johns Hopkins University | University of California, Los Angeles and Kaiser | | | | | | | | | | | | | | | | | | | | | |
| University of Tennessee | Hospital, Los Angeles | | | | | | | | | | | | | | | | | | | | | |
| | Collaborating Inst. in the Collaborative Perinatal Project. | | | | | | | | | | | | | | | | | | | | | |
| LAB/BRANCH Infectious Diseases Branch | | | | | | | | | | | | | | | | | | | | | | |
| SECTION Immunochemistry and Clinical Investigations | | | | | | | | | | | | | | | | | | | | | | |
| INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20014 | | | | | | | | | | | | | | | | | | | | | | |
| TOTAL MANYEARS: <div style="text-align: center; border: 1px solid black; width: 50px; margin: 0 auto;">9</div> | PROFESSIONAL: <div style="text-align: center; border: 1px solid black; width: 50px; margin: 0 auto;">3</div> | OTHER: <div style="text-align: center; border: 1px solid black; width: 50px; margin: 0 auto;">6</div> | | | | | | | | | | | | | | | | | | | | |
| SUMMARY OF WORK (200 words or less - underline keywords) <p> The purpose of this study is to determine insofar as possible the role of <u>perinatal infections</u> in the production of fetal damage. To accomplish this, <u>clinical data</u> and a large number of <u>serial serum specimens</u> have been obtained from the \$58,000 women and their children in the <u>Collaborative Perinatal Project</u>. Now that the project is complete, it is possible to study <u>perinatal infections</u> with three main approaches: 1) <u>Clinical infections</u> 2) <u>Sub-clinical infections</u> detected <u>serologically</u> using <u>abnormals</u> and <u>matched controls</u> and 3) <u>High risk children</u> with <u>elevated IgM levels</u>. Special investigation includes the <u>epidemiology</u> of infections and the frequency of congenital <u>toxoplasmosis</u>. </p> | | | | | | | | | | | | | | | | | | | | | | |

Project Description:

Objectives: The purposes of this study is to determine insofar as possible the role of infections and immunity in the production of abnormal pregnancy outcomes. To accomplish this, 12 collaborating institutions in the Perinatal Research Study plus two special cooperating groups in separate studies have been obtaining specimens of blood and tissue throughout pregnancy, at delivery, post partum, and at set intervals thereafter. These sera are tested to determine the antibody responses of the patients during pregnancy and post partum and then to relate this serological information to the clinical data for the pregnancy and child. In addition, serum specimens from the children were obtained at one year of age from 10,000 study pregnancies. Sera, throat swabs, and urine specimens were also obtained from approximately 5,000 pregnancies. Placental specimens were obtained from 2,500 pregnancies. In special cases when congenital infection is suspected on the basis of clinical or laboratory findings, throat swabs and blood specimens were obtained from the children. Immunoglobulin determinations are performed with the cord blood specimens from the children and specific antibody determinations are also made with these specimens.

Methods Employed: To accomplish this program, blood specimens were obtained from pregnant women at set intervals throughout pregnancy and post partum. Completeness of the sets of sera is determined at the Serum Center of the Infectious Diseases Branch. Data for the 58,000 patients in the Collaborative Perinatal Research Study show that specimens are available from 94.2% of the patients. An average of 5 blood specimens is available for each patient. Each specimen consists of 4 vials with 3 ml of serum in each. For this study then, there are approximately 300,000 serum specimens and almost a million and a half vials of sera. There are an additional 5,000 patients studied to date at the Kaiser Hospital in Los Angeles and approximately 3,000 under study at the Johns Hopkins Medical School in Baltimore, Maryland. All specimens are stored at -20°C until tested and complete filing record concerning basic patient information and the status of the serum available is maintained through a computer system by the Serum Center of the Branch.

In addition to the serum specimens, serial urine and throat specimens were also obtained on a large majority of the patients in the two special studies. These are being studied for direct virus isolation along with swabs obtained from the children at the time of birth.

To date, approximately 62 publications have resulted from the analysis of the data from these studies. The serological method most frequently employed is the complement fixation test with the use of viral antigens. The test is very versatile and can be performed rapidly and provides broad coverage for a great many of the more than 130 viruses which are known to be of importance to man. Antigens were prepared for most of these viruses and tests of specificity were conducted with animal sera. In addition to the complement fixation method, hemagglutination inhibition tests are used for many viral serological determinations. When greater specificity is

needed, neutralization methods are employed. Indirect fluorescence is also used for some of the studies. Virus isolation is conducted with tissue culture of inoculation of experimental animals.

All tests are reproduced completely and a minimum of 95% agreement within two-fold variation is required. All sera showing significant changes in antibody, together with any sera which did not reproduce are tested the third time. We are now completing the study of reported viral, bacterial, and protozoal infections in pregnant women in the study. Serological tests are used to document these reports. The data is then correlated with the pediatric findings. Approximately 2,500 cases of reported viral infections, 3,000 bacterial infections and several hundred protozoal infections, are under investigation. Clinical data is being abstracted, serological tests are being performed in order to document these infections. There are also approximately 1,200 patients identified with a positive serology for syphilis. These are being studied in detail.

A second approach involves a large scale study designed to investigate infection and immunity in relation to 4,000 normal children in the study and 4,000 matched controls. The print-out of abnormal patients has been obtained from the Collaborative Perinatal Research Study (PRB) and this is being reviewed in detail by nurses and physicians from the IDB for more complete information.

From study records, the specific abnormalities under study include abortions; stillbirths, cataracts, congenital heart disease, neonatal deaths, low birthweight (1,000-1,500, 1,500-2,000 grams), IQ below 50, IQ 50-69, enlarged liver, malformations, retarded gross motor development, retarded fine motor development, hearing deficit in both ears, visual impairment, cranial or peripheral nerve damage, cerebral palsy, delayed motor development, hypotonia with poor deep tendon reflexes, non-febrile seizures, dyskinesia and ataxis, hearing deficit in one ear, and elevated bilirubin. The specimens from the mothers of these children and from the children themselves along with carefully matched controls are being studied for antibody to 11 antigens. These antigens include influenza A, rubeola, rubella, mumps, Coxsackie B₂, Coxsackie B₄, varicella, toxoplasmosis, cytomegalovirus, herpes type I, and herpes type II. All of these agents are known or suspected to be responsible for damage in the perinatal period. All laboratory work is being performed under code. The data is being analyzed by Mrs. Gilkeson and Dr. Ellenberg. A second phase of this study will involve 4 additional antigens.

The third approach is to identify the children with elevated IgM levels in the newborn period and then to correlate these findings with pregnancy outcome, clinical performance of the child, and specific serological tests for IgM antibody. Almost 32,000 cord sera have been tested for IgM antibody and approximately 2,000 show elevated levels. These are now being studied in detail.

Major Findings: 1. Maternal Clinical Infections and Pregnancy Outcomes -
The frequency of clinical viral, bacterial, fungal, and parasitic infections

was determined for the 58,828 pregnancies in the Perinatal Research Study. There were 8,180 clinically infected pregnancies (13.9%). The infections included: viral 3,401; bacterial 4,539; fungal 102, and parasitic 138. Influenza "flu"-like illness occurred at the rate of 270 cases /10,000. Specific infections such as mumps, rubella, chickenpox/zoster and measles occurred at rates of 2 to 19 cases per 10,000 pregnancies. Bacterial infections of the kidneys - ureteral - bladder were very frequent (336/10,000 pregnancies). Gonorrhea occurred at a rate of 32/10,000 pregnancies. Pinworm infections were the most frequent of the parasitic infections (12/10,000).

Significance of the Program to the Institute: The use of micro-serological techniques for a large group of new viruses provides an opportunity to investigate the disease caused by viruses which are either difficult to isolate or resistant to evaluation because the clinical effects are delayed until a long time after the infection has subsided. In addition, the availability of new immunologic techniques provides the unique opportunity to detect immunologic deficits and to determine the presence of intrauterine infections on the basis of immunologic response. This data can then be correlated and analyzed as in relation to the possible causes of birth defects. The application of this type of analysis has provided valuable information on the epidemiology of virus infections in relation to abnormal pregnancy outcomes and is constantly giving us new insights into the causes of damage to the central nervous system and possible means of prevention of this and other damage to the developing fetus and newborn.

Proposed Course of the Project: The combined immunologic virologic program will continue during the next year. During that time we will complete the remaining tests for the first phase of the serological studies. Phase two testing will then be initiated using 4 new antigens, including EB and hepatitis.

The three approaches which are being emphasized now include:

1. Completion of the correlation of clinically reported infections in pregnancy with serological findings for the pregnancy, immunologic determinations, and pregnancy outcome. These studies should be completed for the most part in the next fiscal year.
2. A special commitment to perform serological tests on 4,000 abnormal pregnancies and 4,000 matched controls using 11 antigens. The abnormal children have been identified and the laboratory is now approximately 80% of the way through the testing.
3. Special testing of IgM levels from 32,000 cord sera from children in the Collaborative Study and in the cooperative studies. This work provides an index for identifying children with possible congenital infections so that more specific testing can then proceed. These investigations are being tested for specific antibody to toxoplasma, cytomegalovirus, and syphilis. These tests are about complete.

New rapid methods for determining IgM levels and specific IgM antibody will be investigated further. Procedure for removing IgG will be studied further. A special analysis of the epidemiology of infections is in progress and an analysis of toxoplasmosis infections in 23,000 women is being completed.

Publications:

Sever, J.L., Fuccillo, D.A., Ellenberg, J., and Gilkeson, M.R.: Infection and Low Birth Weight in an Industrialized Society. Am. J. Dis. Child. Vol. 219, page 557-558, May 1975.

Kurent, J.E. and Sever, J.L.: Pathogenesis of Intrauterine Infections of the Brain. Biology of Brain Dysfunction, Vol. 3, edited by Gerald E. Gaul, Plenum Press, New York, 1975, pp. 307-341.

Sever, J.L., and Terasaki, P.O.: The Mammalian Fetus edited by E.S.E. Hafez. Charles C. Thomas, Publisher, Springfield, Ill. Feto-Maternal Incompatibility-Cytotoxic Antibodies Against HL-A Tissue Antigens. Jan. 1975, pp. 189-196.

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|---|---|---|-----|-------------------|-------------------------|---------|--|---|
| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 NS 00835-16 ID | | | | | | |
| PERIOD COVERED July 1, 1975 through June 30, 1976 | | | | | | | | |
| TITLE OF PROJECT (80 characters or less) Clinical Investigations with Human Volunteers and Patients Using Viruses, Vaccines and Chemotherapeutic Agents. | | | | | | | | |
| NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT <table style="width: 100%; border: none;"> <tr> <td style="width: 33%;">PI:</td> <td style="width: 33%;">Dr. John L. Sever</td> <td style="width: 33%;">Chief, IDB, IRP, NINCDS</td> </tr> <tr> <td>Others:</td> <td>Dr. Michael Blaese Dr. Maneth Gravell</td> <td>Immunologist, NCI Head, Unit on Neurovirology, IDB, IRP, NINCDS</td> </tr> </table> | | | PI: | Dr. John L. Sever | Chief, IDB, IRP, NINCDS | Others: | Dr. Michael Blaese Dr. Maneth Gravell | Immunologist, NCI Head, Unit on Neurovirology, IDB, IRP, NINCDS |
| PI: | Dr. John L. Sever | Chief, IDB, IRP, NINCDS | | | | | | |
| Others: | Dr. Michael Blaese Dr. Maneth Gravell | Immunologist, NCI Head, Unit on Neurovirology, IDB, IRP, NINCDS | | | | | | |
| COOPERATING UNITS (if any) George Washington University Medical School, Washington, DC Bureau of Prisons, Dept. of Justice, Petersburg Federal Reformatory | | | | | | | | |
| LAB/BRANCH Infectious Diseases Branch | | | | | | | | |
| SECTION Immunochemistry and Clinical Investigations | | | | | | | | |
| INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20014 | | | | | | | | |
| TOTAL MANYEARS: | PROFESSIONAL: | OTHER: | | | | | | |
| 1 | .5 | .5 | | | | | | |
| SUMMARY OF WORK (200 words or less - underline keywords) New <u>prophylactic</u> and <u>therapeutic</u> materials are studied for the prevention and control of <u>infectious diseases</u> in <u>volunteers</u> and <u>patients</u> . Current studies include the evaluation of cellular and humoral immunity in individuals immunized with live rubeola and rubella vaccines. | | | | | | | | |

Project Description:

Objectives: To study new prophylactic and therapeutic materials for the prevention and control of infectious diseases. To study the safety, antigenicity, communicability, and immunogenicity of candidate vaccines.

Methods Employed: Human volunteer studies are conducted in collaboration with the Federal Bureau of Prisons. These studies are reviewed and approved by the Clinical Research Committee and the Medical Board of the National Institutes of Health and the Medical Board of the Federal Bureau of Prisons.

Vaccines and chemotherapeutic agents which appear to warrant further investigations are then utilized in clinical studies of other special patient groups. In addition, clinical studies of epidemic are investigated as a means of identifying the role of infectious agents in the production of perinatal and pediatric disease states.

Major Findings: The immune response of volunteers with rubeola and rubella vaccines have been compared with the immune responses of patients with SSPE and MS. For SSPE there appears to be a serum inhibitor which blocks cellular immunity to measles. The nature of the inhibitor is being investigated.

Transfer factor was given to 8 patients with SSPE. These patients have been followed for 4 years. It appears that some of the patients have not had the expected rapid progression of disease.

Significance of the Program to the Institute: Volunteers studied provide the basic data necessary to evaluate potential chemotherapeutic agents and vaccines. They also provide invaluable information on the course of infection in man. Utilization of this information then in clinical studies provides the necessary bridge to the implementation of therapeutic approaches which originated in the laboratory, are brought through the volunteer studies and then finally are taken to patient population.

Proposed Course of the Project: The additional studies will be performed utilizing rubeola and vaccines to determine antibody and cellular immune responses. Rubeola antibody and cellular immunity in SSPE patients will be studied in detail. Clinical studies will continue on utilization of various drugs for the prevention and treatment of perinatal, acute and chronic infections in man. The drug Levamisole will be studied for the treatment of SSPE.

Publications: None

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| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER <div style="text-align: center; font-size: 1.2em;">Z01 NS 01270-12 ID</div> |
| PERIOD COVERED July 1, 1975 through June 30, 1976 | | |
| TITLE OF PROJECT (80 characters or less) <div style="text-align: center;">Toxoplasmosis: Serological and Clinical Studies</div> | | |
| NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT <div style="display: flex; justify-content: space-between;"> <div style="width: 30%;"> PI: Dr. John L. Sever Others: Dr. Joseph S. Drage </div> <div style="width: 65%;"> Chief, IDB, IRP, NINCDS Chief, Developmental Neurology Branch, NDP, NINCDS </div> </div> | | |
| COOPERATING UNITS (if any) <div style="text-align: center;">None.</div> | | |
| LAB/BRANCH Infectious Diseases Branch | | |
| SECTION Immunochemistry and Clinical Investigations | | |
| INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20014 | | |
| TOTAL MANYEARS: <div style="text-align: center;">0</div> | PROFESSIONAL: <div style="text-align: center;">0</div> | OTHER: <div style="text-align: center;">0</div> |
| SUMMARY OF WORK (200 words or less - underline keywords) <div style="text-align: center; padding-top: 20px;"> This project has been incorporated into Project No. Z01 NS 01992-05 ID. </div> | | |

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|---|---|--|-----|-------------------|-------------------------|---------|----------------|---|--|----------------------|-------------------------|
| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 NS 01503-10 ID | | | | | | | | | |
| PERIOD COVERED: July 1, 1975 through June 30, 1976 | | | | | | | | | | | |
| TITLE OF PROJECT (80 characters or less) Epidemiologic Studies of Perinatal Infections | | | | | | | | | | | |
| NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT <table style="width: 100%; border: none;"> <tr> <td style="width: 15%;">PI:</td> <td style="width: 40%;">Dr. John L. Sever</td> <td style="width: 45%;">Chief, IDB, IRP, NINCDS</td> </tr> <tr> <td>Others:</td> <td>Mrs. Anita Ley</td> <td>Head, Unit on Immunochemistry IDB, IRP, NINCDS</td> </tr> <tr> <td></td> <td>Mrs. Dorothy Edmonds</td> <td>Nurse, IDB, IRP, NINCDS</td> </tr> </table> | | | PI: | Dr. John L. Sever | Chief, IDB, IRP, NINCDS | Others: | Mrs. Anita Ley | Head, Unit on Immunochemistry IDB, IRP, NINCDS | | Mrs. Dorothy Edmonds | Nurse, IDB, IRP, NINCDS |
| PI: | Dr. John L. Sever | Chief, IDB, IRP, NINCDS | | | | | | | | | |
| Others: | Mrs. Anita Ley | Head, Unit on Immunochemistry IDB, IRP, NINCDS | | | | | | | | | |
| | Mrs. Dorothy Edmonds | Nurse, IDB, IRP, NINCDS | | | | | | | | | |
| COOPERATING UNITS (if any) University of Tennessee UCLA and Kaiser Hospital, Los Angeles. | | | | | | | | | | | |
| LAB/BRANCH Infectious Diseases Branch | | | | | | | | | | | |
| SECTION Immunochemistry and Clinical Investigations | | | | | | | | | | | |
| INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20014 | | | | | | | | | | | |
| TOTAL MANYEARS: <div style="text-align: center;">0</div> | PROFESSIONAL: <div style="text-align: center;">0</div> | OTHER: <div style="text-align: center;">0</div> | | | | | | | | | |
| SUMMARY OF WORK (200 words or less - underline keywords) This project has been incorporated into Project No. Z01 NS 01992-05 ID. | | | | | | | | | | | |

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| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 NS 01981-05 ID |
| PERIOD COVERED July 1, 1975 through June 30, 1976 | | |
| TITLE OF PROJECT (80 characters or less) Immunoglobulin M and Congenital Infection | | |
| NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT PI: Dr. John L. Sever Chief, IDB, IRP, NINCDS Others: Mrs. Anita Ley Head, Unit on Immunochemistry, IDB IRP, NINCDS | | |
| COOPERATING UNITS (if any) University of Tennessee | | |
| LAB/BRANCH Infectious Diseases Branch | | |
| SECTION Immunochemistry and Clinical Investigations. | | |
| INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20014 | | |
| TOTAL MANYEARS: .5 | PROFESSIONAL: .25 | OTHER: .25 |
| SUMMARY OF WORK (200 words or less - underline keywords) This project has been incorporated into project Z01 NS 01992-05 ID. | | |

PERIOD COVERED

July 1, 1975 through June 30, 1976

TITLE OF PROJECT (80 characters or less)

Immunological Studies of Chronic Infections

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

| | | |
|---------|--------------------|---|
| PI: | Dr. John L. Sever | Chief, IDB, IRP, NINCDS |
| Others: | Dr. David Fuccillo | Head, Section on Neurovirology, IDB, IRP, NINCDS |
| | Dr. David Madden | Head, Unit on Microbiology, IDB, IRP, NINCDS |
| | Dr. William London | Head, Section on Experimental Pathology, IDB, IRP, NINCDS |
| | Mrs. Anita Ley | Head, Unit on Immunochemistry, IDB, IRP, NINCDS |

COOPERATING UNITS (if any)

University of Vermont

LAB/BRANCH

Infectious Diseases Branch

SECTION

Immunochemistry and Clinical Investigations

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:

0

PROFESSIONAL:

OTHER:

SUMMARY OF WORK (200 words or less - underline keywords)

This project was incorporated into project Z01 NS 02038-04 ID.

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| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 NS 01992-05 ID |
|--|---|--|

PERIOD COVERED
July 1, 1975 through June 30, 1976

TITLE OF PROJECT (80 characters or less)

High Risk Pregnancies and Perinatal Infections

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

| | | |
|---------|----------------------|---|
| PI: | Dr. John L. Sever | Chief, IDB, IRP, NINCDS |
| Others: | Mrs. Anita Ley | Head, Unit on Immunochemistry IDB, IRP, NINCDS |
| | Mrs. Dorothy Edmonds | Nurse, IDB, IRP, NINCDS |

COOPERATING UNITS (if any)

University of Tennessee
UCLA and Kaiser Hospital, Los Angeles.
George Washington University Hospital, Washington, DC

LAB/BRANCH

Infectious Diseases Branch

SECTION

Immunochemistry and Clinical Investigations

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:

3

PROFESSIONAL:

2

OTHER:

1

SUMMARY OF WORK (200 words or less - underline keywords)

The purpose of this investigation is to study high risk pregnancies in relation to perinatal infections. Cooperating units work with the Infectious Diseases Branch to study newborns in high risk nurseries. Serum, IgM volumes, plus clinical findings are being used to identify infected infants at risk for perinatal damage. Specific tests are then developed and applied for identification of the infection. Amniotic fluid specimens are studied for micro-organisms and new alpha fetoprotein tests are being investigated to detect CNS malformations. New methods for detecting infections are being investigated.

Project Numbers 01270-12, 01503-10, 01981-05, 01993-05, 02067-03 and 02135-02 have been incorporated into Project No. Z01 NS 01992-05 shown above.

Project Description:

Objectives: The purpose of this investigation is to study high risk pregnancies in relation to perinatal infections. To accomplish this, a special study was developed at the newborn nursery at the University of Tennessee. This is a high risk nursery where infants from the Memphis Metropolitan area are brought for special newborn care and procedures. Serial blood specimens are obtained and tested for immunoglobulin levels as a means of identifying those children with possible intrauterine infection. In addition, other laboratory tests and specimens are obtained to further define the infections which may be present. This study focuses on a group of infants which is most likely to have difficulties in the newborn period and is most frequently involved with infections. In a second study at the Kaiser Hospital, Los Angeles, we are investigating the role of infection in abortions, infertility, and CNS disease in newborns. A third study at the George Washington University Hospital, amniotic fluid sample and newborns are tested for antibody and infection. A new limulus test is being evaluated for detection of bacterial infection and alpha fetoprotein levels are being determined for identification of CNS defects.

Methods Employed: For the study at the University of Tennessee, serial heel prick blood specimens are obtained during the first weeks of life. These specimens are tested for IgM levels using radial immunodiffusion techniques. Infants showing elevated IgM levels are then further tested using venous blood specimens, throat swabs, anal swabs and other tissue specimens. These latter specimens are forwarded to the laboratories of the Infectious Diseases Branch for study. Virus isolation procedures and specific antibody tests are used. The data is then analyzed in relation to the clinical findings in the child and treatment is instituted wherever appropriate.

For the study at the Kaiser Hospital, 4,000 pregnant women have been studied to determine the frequency of infection and the effects of the infections on the children. Additional studies of cytomegalovirus by direct isolation of the virus are being conducted. A special study of 100 women attending an infertility clinic is in progress.

Major Findings: At the George Washington University Hospital a new combined virology-bacteriology study is being conducted using amniotic fluid and newborn specimens to detect infection. At the University of Tennessee approximately 1,500 children have been studied this year. We find that an unusually high rate of IgM elevation in this population exists. Investigations are in progress to define the specific organisms which may be responsible for the congenital infections. At the Kaiser study, higher rates of infection with toxoplasmosis and cytomegalovirus have been found among the women in the infertility groups.

The Significance of the Program to the Institute: In our effort to identify the causes of neurological and other damage to the developing child, these populations provide unique opportunities of studying high risk pregnancies

and thus developing a great deal of information with a minimal amount of study and testing. We are focusing in on populations which are at high risk for congenital infections.

Proposed Course of the Project: The study at the University of Tennessee will continue during which a total of 6,000 patients will be studied. Combined serological and virus isolation techniques will be used. The effect of Group B streptococcal infections will be investigated. At the Kaiser-UCLA study, analysis of data will be completed. The study at George Washington University Hospital will utilize new methods such as the limulus lysate test to detect perinatal and in utero infections. Alpha fetoprotein tests will be included.

Publications:

Sever, J.L., Kapikian, A.Z., Finestone, S., Purcell, R., and Gilkeson, M.R.: Hepatitis A and Down's Syndrome: Lack of an Association. J. Inf. Dis., July 19, 1976 (in press).

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| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 NS 01993-05 ID |
| PERIOD COVERED July 1, 1975 through June 30, 1976 | | |
| TITLE OF PROJECT (80 characters or less) Amniotic Fluid, Fetal Infection and Antibody | | |
| NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT PI: Dr. John L. Sever Chief, IDB, IRP, NINCDS Others: Dr. John Larsen Guest Worker, IDB, IRP, NINCDS | | |
| COOPERATING UNITS (if any) University of Oklahoma George Washington University Hospital, Washington, DC | | |
| LAB/BRANCH Infectious Diseases Branch | | |
| SECTION Immunochemistry and Clinical Investigations | | |
| INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20014 | | |
| TOTAL MANYEARS: 0 | PROFESSIONAL: | OTHER: |
| SUMMARY OF WORK (200 words or less - underline keywords) This project has been incorporated into project No. Z01 NS 01992-05 ID | | |

PERIOD COVERED

July 1, 1975 through June 30, 1976

TITLE OF PROJECT (80 characters or less)

Clinical Studies of Chronic Infections of the Central Nervous System.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

| | | |
|---------|----------------------|--|
| P.I. | Dr. John L. Sever | Chief, IDB, IRP, NINCDS |
| Others: | Dr. David Fuccillo | Head, Section on Neurovirology, IDB, IRP, NINCDS |
| | Dr. Jonas Ellenberg | Asst. Head, Section on Mathematical Statistics, OBE, OD, NINCDS |
| | Dr. Ronald Kartzinel | Clinical Associate, Laboratory of Neuro- pharmacology, NINCDS |

COOPERATING UNITS (if any)

University of Tennessee
VA Hospital, Washington, DC
University of Vermont

LAB/BRANCH

Infectious Diseases Branch

SECTION

Immunochemistry and Clinical Investigations

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:

0

PROFESSIONAL:

OTHER:

SUMMARY OF WORK (200 words or less - underline keywords)

This project was incorporated into project Z01 NS 02038-04 ID

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| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | | U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | | PROJECT NUMBER Z01 NS 02038-04 ID | |
| PERIOD COVERED July 1, 1975 through June 30, 1976 | | | | | |
| TITLE OF PROJECT (80 characters or less) Combined Clinical, Viral and Immunological Investigations of Chronic Diseases of the Central Nervous System | | | | | |
| NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT | | | | | |
| PI: | | Dr. John L. Sever | | Chief, IDB, IRP, NINCDS | |
| Others: | | Dr. Maneth Gravell | | Head, Unit on Neurovirology Diseases IDB, IRP, NINCDS | |
| | | Dr. Monique Dubois-Dalcq | | Head, Unit on Electron Microscopy IDB, IRP, NINCDS | |
| | | Dr. David Fuccillo | | Head, Section on Neurovirology IDB, IRP, NINCDS | |
| | | Dr. Roswell Eldridge | | Head, Section on Neurogenetics, IDB, IRP, NINCDS | |
| | | Mrs. Anita Ley | | Head, Unit on Immunochemistry IDB, IRP, NINCDS | |
| COOPERATING UNITS (if any) | | | | | |
| University of Tennessee | | Georgetown University Medical School, | | | |
| University of Vermont | | Washington, DC | | | |
| VA Hospital, Washington, DC | | | | | |
| LAB/BRANCH Infectious Diseases Branch | | | | | |
| SECTION Immunochemistry and Clinical Investigations | | | | | |
| INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20014 | | | | | |
| TOTAL MANYEARS: | | PROFESSIONAL: | | OTHER: | |
| 3 | | 2 | | 1 | |
| SUMMARY OF WORK (200 words or less - underline keywords) | | | | | |
| <p><u>Clinical</u> and <u>Laboratory</u> Studies are conducted to determine if infection, immunity and/or genetics are responsible for chronic diseases of the central nervous system. Current studies include <u>Multiple Sclerosis</u>, <u>Subacute Sclerosing Panencephalitis</u>, <u>Amyotrophic Lateral Sclerosis</u> and <u>Parkinson's Disease</u>. Combined clinical data, genetic information, HLA and MLC typing, virus serology and <u>virus isolation studies</u> are obtained in these investigations.</p> | | | | | |
| <p>Project Numbers 01994-05 and 01994-05 have been incorporated into Project No. Z01 NS 02038-04 ID shown above.</p> | | | | | |

Project Description:

Objectives: Clinical and laboratory studies are being conducted on chronic infections of the central nervous system. During this year, the investigations have primarily centered on multiple sclerosis, subacute sclerosing panencephalitis, amyotrophic lateral sclerosis and Parkinson's disease. These studies have epidemiological, serological, viral and therapeutic components. They involve collaboration of a number of groups through the United States.

Methods Employed:

Multiple Sclerosis patients and tissues are obtained from a number of collaborators throughout the world. New tissue culture methods and electron microscopic techniques are used in these studies as well as genetic and cellular immune tests.

A registry for subacute sclerosing panencephalitis was initiated as a joint effort with Dr. J.T. Jabbour at the University of Tennessee. This registry now has data for more than 400 cases of SSPE. The reporting of patients is being continued to determine if there will be a change in the number of cases per year related to the widespread use of measles vaccine. This investigation has provided us with opportunities to study unusual patients such as one individual with hypogammaglobulinemia, as well as SSPE in one of two identical twins. Additional studies have been conducted on the epidemiology of multiple sclerosis. Serum and spinal fluid specimens are being tested for antibody to a variety of viruses. Tissue specimens are studied for the presence of viruses using tissue culture methods and electron microscopy. Similar studies of amyotrophic lateral sclerosis and Parkinson's disease are in progress.

Major Findings: Analysis of the data for patients with SSPE has brought out a number of new findings: 1) The "incubation" period between measles and onset of disease is 6.5 years. 2) This interval is surprising-constant for all ages of measles (1 year through 6 years). 3) The administration of measles vaccine after measles did not significantly change this interval. 4) Subacute sclerosing panencephalitis (SSPE) is rarely seen in Blacks in this study population although most patients come from rural South United States.

Patients with multiple sclerosis are being tested for cellular immunity in relation to measles and other viruses. ALS patients are being studied for polio and other virus antibodies. Patients with Parkinson's disease are being tested for antibody to 11 viruses.

Significance of the Program to the Institute: Clinical and laboratory studies of MS, SSPE, ALS and Parkinson's disease permit direct investigation of the possible causes of these diseases, and provide us with an opportunity to study unique "experiments" of nature which often provide very valuable insight into the diseases process. These studies are designed to take advantage of both the epidemiology as well as the direct

laboratory approaches to the problems of chronic infections of the CNS.

Proposed Course of the Project: Studies of MS patients will include combined genetic, HLA, DW2, cellular immune virus isolation investigation. We are working extensively with the Multiple Sclerosis Associated Agent reported to Dr. Carp and confirmed by Dr. Henle. We are attempting to reproduce their findings. We plan to continue this SSPE registry to determine the effect of the widespread measles immunization program, which has been in effect in the United States for more than 10 years. If the vaccine influences the rate of SSPE, this change should occur in the next few years. We will extend our serology and genetic studies of ALS and Parkinson's disease.

Publications:

Sever, J.L.: Perspectives in Multiple Sclerosis - 1975 - Introduction Neurology, Minneapolis, Vol. 25, No. 5, May 1975.

Sever, J.L.: The current Status of Viruses in Multiple Sclerosis. Current Reports in Neurology, Vol. 1, No. 1, page 2-3, 1975.

Oldstone, M.B.A., Bokisch, V.A., Dixon, F.J., and Barbosa, L.H., Fuccillo, D.A., and Sever, J.L.: Subacute Sclerosing Panencephalitis: Destruction of Human Brain Cells by Antibody and Complement in an Autologous System. Clinical Immunology and Immunopathology 4, 52-58 (1975).

Kurent, J.E., Sever, J.L., Terasaki, P.L.: HL-A W29 and Subacute Sclerosing Panencephalitis. The Lancet 927-928, April 19, 1975.

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| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 NS 02067-03 ID |
| PERIOD COVERED July 1, 1975 through June 30, 1976 | | |
| TITLE OF PROJECT (80 characters or less) Antenatal Diagnosis of Anencephaly and Spina Bifida | | |
| NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT | | |
| PI: | Dr. John L. Sever | Chief, IDB, IRP, NINCDS |
| Others: | Mrs. Dorothy Edmonds Dr. Thomas Waldman | Nurse, IDB, IRP, NINCDS Chief, Metabolism Branch, NCI |
| COOPERATING UNITS (if any) Johns Hopkins University Hospital | | |
| LAB/BRANCH Infectious Diseases Branch | | |
| SECTION Immunochemistry and Clinical Investigations | | |
| INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20014 | | |
| TOTAL MANYEARS: <div style="text-align: center;">0</div> | PROFESSIONAL: | OTHER: |
| SUMMARY OF WORK (200 words or less - underline keywords) This project has been incorporated into project Z01 NS 01992-05 ID. | | |

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| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 NS 02135-02-ID |
| PERIOD COVERED July 1, 1975 through June 30, 1976 | | |
| TITLE OF PROJECT (80 characters or less) Rapid Diagnosis of Meningitis | | |
| NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT PI: Dr. John L. Sever Chief, IDB, IRP, NINCDS Others: Dr. John Larsen Guest Worker, IDB, IRP, NINCDS | | |
| COOPERATING UNITS (if any) George Washington University Hospital, Washington, DC Children's Hospital, Washington, DC | | |
| LAB/BRANCH Infectious Diseases Branch | | |
| SECTION Immunochemistry and Clinical Investigations | | |
| INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20014 | | |
| TOTAL MANYEARS: 0 | PROFESSIONAL: | OTHER: |
| SUMMARY OF WORK (200 words or less - underline keywords) This project has been incorporated into project Z01 NS 01992-05 ID. | | |

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| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 NS 01731-08 ID |
| PERIOD COVERED July 1, 1975 to June 30, 1976 | | |
| TITLE OF PROJECT (80 characters or less) Isolation of Infective Agents from Chronic Diseases | | |
| NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT | | |
| PI: Dr. Maneth Gravell Dr. Monique Dubois- Dalcq Other: Mrs. Rebecca Hamilton Mr. Leonard Moore | Head, Unit on Neurovirology Diseases Head, Sec. Electron Microscopy Biologist Biological Lab Technician | IDB, IRP, NINCDS IDB, IRP, NINCDS IDB, IRP, NINCDS IDB, IRP, NINCDS |
| COOPERATING UNITS (if any) None | | |
| LAB/BRANCH Infectious Diseases Branch | | |
| SECTION Neurovirology | | |
| INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Md. 20014 | | |
| TOTAL MANYEARS: 1.8 | PROFESSIONAL: 0.4 | OTHER: 1.4 |
| SUMMARY OF WORK (200 words or less - underline keywords) Two independent laboratories recently reported the association of an <u>infectious virus-sized agent</u> with <u>multiple sclerosis (MS)</u> . The agent has been found in <u>serum, cerebrospinal fluid, brain, spleen, kidney and lymph nodes</u> from <u>confirmed MS patients</u> but not from <u>controls</u> . Our current <u>major effort</u> on MS is devoted to <u>confirming this work</u> . <u>Results</u> of these studies <u>to date</u> have been <u>inconclusive</u> . | | |

Project Description:

Objectives: To determine whether persistent viral infections are the cause of chronic neurological diseases such as multiple sclerosis (MS).

Methods Employed: Brain specimens from well documented cases of MS were homogenized as 10% suspensions, clarified, quick frozen and stored at -70°C . PAM cells, a line of transformed mouse cells, were inoculated with 10% homogenates of MS brain or control non-MS brain. Growth rates of these cultures were compared to uninoculated PAM controls. Cultures inoculated with MS material have been reported to have a decreased growth rate compared to controls.

Tissues from patients with neurological diseases were examined by electron microscopy, fluorescent microscopy, and whenever possible, cultured in vitro. In vitro cultured cells were submitted to virologic and serologic assays, inoculated into experimental animals and co-cultivated with human diploid or heteroid cell lines in efforts to isolate viral agents.

Brain tissue from mice inoculated with human MS materials was minced and cultured in vitro in attempts to establish cell lines which may contain a multiple sclerosis-associated agent.

Major Findings: Two independent laboratories recently reported the association of an infectious virus-sized agent with multiple sclerosis. The agent has been found in serum, cerebrospinal fluid, brain, spleen, kidney and lymph nodes from confirmed multiple sclerosis patients but not from controls. Our current major effort is devoted to confirming this work. One assay we have focused on is called the "PAM" cell assay, an assay in which "PAM" cells treated with MS materials have a reduced growth rate compared to controls. Although results of these studies have at times been encouraging, they have been complicated by mycoplasma contamination. Work is in progress to decontaminate the PAM cells of mycoplasma.

It has also been reported that brain tissue of mice inoculated with MS material is readily established as permanent cell lines when cultured in vitro. These permanent cell lines produce an MS-associated agent as determined by the mouse "PMN" depression assay. Brain culture have been established from mice inoculated with normal or MS material; however, they have not been in culture sufficient time to determine whether permanent cell lines have been established and whether these cells produce the MS-associated agent.

Work is in progress to establish cultures from human MS material.

Significance of the Program to the Institute: Multiple sclerosis (MS) is one of the major disorders of the human central nervous system. No good diagnostic laboratory tests are currently available for MS, although cerebrospinal fluids of MS patients frequently contain elevated levels of protein, particularly oligoclonal IgG. The association of an infectious agent with multiple sclerosis and the development of assays to detect this agent could lead to a more complete understanding of the agent and to improved methods of prevention and treatment of the disease.

Proposed Course of the Project: Initially, major emphasis will be placed on developing sensitive and reproducible assays for the MS-associated agent. If the first phase of the project is successful, work will be done to characterize the MS-associated agent biologically, physically and chemically. Studies will also be initiated to better understand the epidemiology of MS. Attempts to establish animal models of MS will also be made.

Publications: None

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| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 NS 01732-08 ID |
| PERIOD COVERED July 1, 1975 to June 30, 1976 | | |
| TITLE OF PROJECT (80 characters or less) Investigations of Non-Viral Agents in Perinatal and Neurological Diseases. | | |
| NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT | | |
| PI: Dr. David L. Madden Head, Unit on Microbiology IDB, IRP, NINCDS | | |
| Other: Dr. John L. Sever Chief, IDB, IRP, NINCDS IDB, IRP, NINCDS Dr. David Fuccillo Head, Sec. on Neurovirology IDB, IRP, NINCDS Mrs. Aurella Krezlewicz Microbiologist IDB, IRP, NINCDS Ms. Janet Mattson Microbiologist IDB, IRP, NINCDS | | |
| COOPERATING UNITS (if any) George Washington University Hospital | | |
| LAB/BRANCH Infectious Diseases Branch | | |
| SECTION Neurovirology | | |
| INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Md. 20014 | | |
| TOTAL MANYEARS: .75 | PROFESSIONAL: .25 | OTHER: .50 |
| SUMMARY OF WORK (200 words or less - underline keywords) The role of <u>mycoplasma</u> , chylamidia and other non-viral agents in diseases of man particularly those associated with <u>perinatal disease</u> , <u>infertility</u> or neurological disease has been studied. Reproductive tract samples from men studied for presence of these organisms, classical strain mycoplasma, T-strain mycoplasma and chylamidia were recovered from both men without clinical disease and from those with genital tract infections. Routine monitoring of culture from experimental viral studies and efforts to develop new techniques to monitor tissue culture for non-viral agents have been continued. | | |

Project Description:

Objectives: To study the role of mycoplasma, chylamidia and other non-viral agents in disease of man particularly those associated with perinatal disease, infertility, or neurological disease. To develop animal models and to determine the pathogenesis of these diseases. To develop new tests for identification of mycoplasma.

Method Employed: Urine samples and small pieces of vas deferens were collected from men undergoing vasectomy. Urine samples, urethral swabs and exudate from men with chronic nonbacterial urethritis were collected. These samples were cultured for presence of mycoplasma and chylamidia and positive cultures were identified by standard techniques.

Small pieces of biopsy material from a variety of neurological diseases and secondary tissue cultures are also being studied for presence of mycoplasma. These include such diseases as Subacute Sclerosing Panencephalitis, Alateral Sclerosis, Multiple Sclerosis, and Creutzfeldt-Jakob disease. These tissues are being cultured in standard mycoplasma media, and the positive isolated cultures are being identified by standard techniques.

Virus preparations and antigens used in cellular immune studies have been examined for presence of mycoplasma. Attempts to determine immune responses of patients to mycoplasma antigens have been attempted.

Major Findings: The study on occurrence of mycoplasma and chylamidia have been continued. Classical strain mycoplasma (Mycoplasma hominis) were recovered from about 10% of the males studied in the Vasectomy Clinic at the National Naval Medical Center. T-strain mycoplasma were recovered from about 20% of these patients. An intensive study to define the occurrence of mycoplasma and chylamidia as well as other viruses (Dr. Fuccillo and Bacteria Dr. Klousia) was undertaken. 100 patients with some bacterial urethritis and 100 controls were selected. Mycoplasma hominis was isolated from 14% of the patients and 12% of the controls. T-strain mycoplasma were isolated from 25% of the patients and 20% of the controls. Chylamidia studies have not yet been completed. The frequency of mycoplasma in patients with nonbacterial urethritis did not seem to be significantly higher than in controls without urinary infection. In both patients and controls there seemed to be a direct correlation between sexual activity and occurrence of mycoplasma. Studies of tissue culture lines, seed viruses used to produce antigens or antibodies and primary isolation tissue indicate that mycoplasma often occur as contaminants when least expected. Continual monitoring of common reagents used in infectious Disease studies is necessary to prevent these contaminating agents from producing interfering results.

Significance of the Program to the Institute : A program devoted to studying the effects of mycoplasma, chylamidia and other non-viral agents in various diseases complements the virological studies currently being done. This study and its support given to other investigators may help to more accurately define the role of various agents in disease.

Proposed Course of the Projects: Studies to determine the effect of mycoplasma isolated from viral antigens on cellular immunity are underway. Correlation of these results with results obtained from mycoplasma contaminated antigens and mycoplasma-free antigens may provide information about mechanisms of cellular immunity for diagnosis of disease. Efforts are underway to initiate a study to determine the frequency of mycoplasma in amniotic fluid and in infertility and repeat abortion. Attempts to define the role of mycoplasma in neurological diseases will be continued. Continued efforts will be made to apply new techniques in the identification of mycoplasma and mycoplasmal disease.

Publications: None

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| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 NS 01903-06 ID |
| PERIOD COVERED July 1, 1975 to June 30, 1976 | | |
| TITLE OF PROJECT (80 characters or less) Investigation of the Etiology and Effect of Hepatitis A and B in the Perinatal Period. | | |
| NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT PI: Dr. David L. Madden Head, Unit on Microbiology IDB, IRP, NINCDS | | |
| COOPERATING UNITS (if any) Lynchburg Training School and Hospital Electronucleonics, Inc. | | |
| LAB/BRANCH Infectious Diseases Branch, IRP | | |
| SECTION Neurovirology | | |
| INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Md. 20014 | | |
| TOTAL MANYEARS: 0 | PROFESSIONAL: 0 | OTHER: 0 |
| SUMMARY OF WORK (200 words or less - underline keywords) With the publication of the two manuscripts listed this project was terminated. Radioimmunoprecipitation and passive hemmagglutination tests were used to determine hepatitis B antigen and antibody. Epidemiological studies in institutionalized mentally retarded patients have shown that hepatitis B virus infection is an important disease. However, no correlation of hepatitis B infection and cause of mental retardation could be identified. | | |

Project Description:

Objectives: To determine the etiology of Australia antigen associated (serum) and infectious hepatitis. To determine the relationship of hepatitis/congenital jaundice and postnatal jaundice. To develop animal models and new diagnostic tests for these diseases.

Methods Employed: A large epidemic of infectious hepatitis (Hepatitis A) occurred in the Lynchburg Training School and Hospital during the summer of 1970. Serial samples of feces and serum were obtained from many patients prior to the development of the disease, at the time of acute disease and post-infection. Serum samples have been obtained from these patients at 6, 12, 18, 24, 36 and 48 months post-infection. Complement fixation and radioimmunoprecipitation tests have been used to detect hepatitis B antigen in these patients. Radioimmunoprecipitation and passive hemagglutination tests have been used to determine antibody levels. In cooperation with Electronucleonics 12 samples of serum and feces have been examined by electron microscopic and immunoelectron microscopic techniques for presence of hepatitis A antigen.

Major Findings: Epidemiological studies in institutionalized mentally retarded patients have shown that Hepatitis B virus infection, as determined by presence of either HB surface antigen or HB surface antibody, is an important disease of institutionalized patients. However, no correlation of Hepatitis B infection and cause of mental retardation could be identified.

Epidemiological studies on hepatitis A in institutionalized patients have been continued. Typical hepatitis A particles have been demonstrated in 4 of 12 fecal samples studied. These samples were collected at the time of peak SGPT rise. Serological studies (immunoelectron microscopic techniques) on patients serum who did not develop hepatitis A in the outbreak indicated that they had circulating antibodies prior to the epidemic.

Significance of the Program to the Institute : Hepatitis in pregnancy has been associated with neonatal jaundice, Down's Syndrome and other forms of mental retardation. Among the institutionalized mentally retarded patients those with trisomy 21 Karyotypes have a higher rate of chronic hepatitis B antigenemia than others. This study has provided additional depth in determining the role of infectious agents in perinatal disease and mental retardation. Data obtained from this study has clarified the association of hepatitis with various perinatal diseases and mental retardation.

Proposed Course of the Project: Termination

Publications:

Madden, D.L., Dietzman, D.E., Matthew, E.B., Sever, J.L., Lander, J.J., Purcell, R.H., Rostafinski, M. and Mata, A.: Epidemiology of Hepatitis B Virus in an Institution for Mentally Retarded Persons. Amer. J. Ment. Defic. Vol. 80, No. 4, p. 369-375, 1976.

Madden, D.L., Matthew, E.B., Dietzman, D.E., Purcell, R.H., Sever, J.L., Rostafinski, M. and Mata, A.: Hepatitis and Down's Syndrome. Amer. J. Ment. Defic. Vol. 80, No. 4, p. 401-406, 1976.

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| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 NS 01982-05 ID |
| PERIOD COVERED July 1, 1975 through June 30, 1976 | | |
| TITLE OF PROJECT (80 characters or less) Delayed Hypersensitivity in Chronic Viral Diseases | | |
| NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT | | |
| PI: | Dr. David A. Fuccillo | Head Section on Neurovirology IDB, IRP, NINCDS |
| Others: | Dr. David Madden | Head Unit on Microbiology IDB, IRP, NINCDS |
| | Mrs. Renee Traub | Microbiologist Section on Neurovirology, IDB, IRP, NINCDS |
| COOPERATING UNITS (if any) Georgetown University School of Medicine, Washington, DC Brooke Army Medical Center, Fort Sam Houston, Texas Microbiological Associates, Bethesda, Maryland | | |
| LAB/BRANCH Infectious Diseases Branch | | |
| SECTION Neurovirology | | |
| INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20014 | | |
| TOTAL MANYEARS: | PROFESSIONAL: | OTHER: |
| 1 | 0.5 | 0.5 |
| SUMMARY OF WORK (200 words or less - underline keywords) | | |
| <p>In the last few years, intensive studies have been conducted on the question of altered immune responses and viruses in MS. Our studies have focused on specific immune responses to viruses in MS patients and carefully matched controls. In a large study of 53 MS patients and controls we have found no impaired response against measles using three different tests. These tests included <u>direct migration inhibition</u>, <u>complement mediated cytotoxicity</u> and <u>lymphocytic mediated cytotoxicity</u>.</p> | | |

Project Description:

Objectives: To determine the role of delayed hypersensitivity (cellular immunity) in chronic viral infections.

Methods Employed: A radioactive aliquot transfer technique for cell mediated immunity was developed which will increase the number of specimens that can be done at one time.

A lymphotoxin assay and an antibody complement mediated test for rubella and rubeola have been developed. Other tests for CMV and Herpes are being worked on at the present time.

A rubella chronically infected cell line was found to be very useful for the lymphotoxin assay. This assay was developed and is being tested against children who have developed an SSPE like disease, but are apparently congenitally rubella infected individuals.

A rubeola chronically infected cell line was developed. This system is now being used in the study of SSPE and MS patients to determine their cellular immune capabilities against this virus.

A leukocyte inhibitory factor test (LIF) was established in our laboratory to study the capabilities of MS patients and controls against a number of viral antigens.

Major Findings: The cellular immunity of 53 MS patients and very rigidly selected controls were studied. The MS patients recognized measles antigens as well as controls. The LIF test, using CMV and herpes antigens, also demonstrated no impairment of MS patients cellular immunity. A lymphocytotoxic test using a rubeola target cell demonstrated the presence of an inhibitor in the spinal fluids of some SSPE patients.

Significance of the Program to the Institute: Prior to the development of the above tests, no reliable tests for measuring cellular immunity in humans to viral antigens existed. Antibody tests to viral antigens have been developed and used in this and other laboratories for many years. This represents a test for only part of the body's immune response. The development of lymphotoxin and the LIF assays into a clinically useable test for virus infection now allows both forms of the body's protective immune mechanism, humoral and cellular, to be tested simultaneously. Treatment of patients found to have any defects may then be possible.

Proposed Course of the Project: To complete the above mentioned specific projects and to develop additional tests to elucidate how the cellular immune system is involved with other viral diseases, especially those that cause congenital infection. Congenitally infected infants excrete virus in the presence of high antibody titers, possibly indicating that their cellular immune system is impaired. The effect of complement as well as the function of cells other than lymphocytes can be studied with this

system. These tests will also be used to determine the cellular immune capabilities of patients with MS and other neurological diseases. These tests will also be used to detect cell surface changes of infected cells. The Carp agent in infected Pam cells will be tested as a target cell for cellular immune studies with MS patients lymphocytes.

Publications: Steele, R.W., Fuccillo, D.A., Hensen, S.A., Vincent, M.M., and Bellanti, J.A.: Specific Inhibition Factors of Cellular Immunity in Children with SSPE. Jour. of Ped. 88, 52-62, 1976.

Bellanti, J.A., Peters, S.M., Steele, R.W., Vincent, M.M., Hensen, S.A., Fuccillo, D.A., Hurtado, R.C., Thong, Y.H. and Rola-Pleszczynski, M.: Studies on Local Cell-Mediated Immunity. Neter, E. and Milgram, F., (Eds) The Immune System and Infectious Diseases. 4th International Convocation on Immunology, Buffalo, New York, pp. 347-365, Karger, Basel, 1975.

Thong, Y.H., Hensen, S.A., Vincent, M.M., Fuccillo, D.A., Stiles, W.A., and Bellanti, J.A.: Use of Cryopreserved Virus-Infected Target Cells in a Lymphocytotoxicity ⁵¹Cr Release Microassay for Cell-Mediated Immunity to Cytomegalovirus. Infection and Immunity, Vol. 13, No. 2, p. 643-645, Feb. 1976.

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| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | | U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | | PROJECT NUMBER Z01 NS 01983-05 ID |
| PERIOD COVERED July 1, 1975 through June 30, 1976 | | | | |
| TITLE OF PROJECT (80 characters or less) Chronic Viral Infections | | | | |
| NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT | | | | |
| PI: | Dr. David A. Fuccillo | Head | Section on Neurovirology IDB, IRP, NINCDS | |
| Others: | Dr. John L. Sever | Chief | Infectious Diseases Branch IRP, NINCDS | |
| | Dr. William T. London | Head | Section on Experimental Pathology, IDB, IRP, NINCDS | |
| | Dr. David L. Madden | Head | Unit on Microbiology, IDB IRP, NINCDS | |
| | Mrs. Flora Moder | Microbiologist | Section on Neurovirology Unit on Acute Viral Dis. IDB, IRP, NINCDS | |
| | Mrs. Renee Traub | Microbiologist | Section on Neurovirology IDB, IRP, NINCDS | |
| COOPERATING UNITS (if any) Microbiological Associates, Bethesda, Md. Univ. of Texas Medical Harvard Medical School, Boston, Massachusetts School, San Antonio, Texas UCLA Center for Health Sciences, Los Angeles, Calif. George Washington Univ. Medical School, Wash. DC | | | | |
| LAB/BRANCH Infectious Diseases Branch | | | | |
| SECTION Neurovirology | | | | |
| INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20014 | | | | |
| TOTAL MANYEARS: 2 | | PROFESSIONAL: 1 | | OTHER: 1 |
| SUMMARY OF WORK (200 words or less - underline keywords) Radioimmune assays were perfected for Herpes I and II, CMV, EBV and Varicella viruses which all cause <u>chronic viral infections</u> in humans. With this sensitive assay available, it should now be possible to determine slight changes in the <u>immune response</u> to any one of these viruses. | | | | |

Project Description:

Objectives: The main objectives of this project is to establish the clinical and biological significance of the two different strains of herpes simplex virus (Type I, "oral" and Type II, "genital") cytomegalovirus, varicella, in the causation of human disease including carcinoma, ulcerative colitis, Bells' palsy, facial paralysis, herpes zoster and chronic neurological diseases.

Methods Employed: The principal methods employed are: (1) the indirect hemagglutination test, by which type specific herpesvirus antibody is identified; (2) mass serological surveys of materials from selected patients with special disease entities; (3) virus isolation, titration, and characterization procedures; (4) monolayer cultivation of tissue with cocultivation; (5) fluorescent antibody studies of monolayer cultures for the presence of latent infection.

Major Findings: In collaboration with Dr. Smith, RIA tests were perfected for Herpes, CMV, toxoplasma and varicella. A number of serial serum specimens were collected by Dr. Johnson on primary herpes infections. Comparison serology was found to be the most specific test for Herpes Type II. In collaboration with Dr. Gitnick, a number of sera from ulcerative colitis patients were serologically studied. CMV titers were still higher with the ulcerative colitis patients. Sera will be screened against a new agent recently isolated. In collaboration with Dr. Klousia, approximately 150 chronic urethritis and controls were studied for mycoplasma, Herpes, CMV and Trachoma. Results are being prepared for publication.

A group of mothers who had children with brain tumors were studied to determine if SV₄₀ PML (JC)antibodies were present in their sera at any greater levels as compared to the controls. No significant differences were found. These investigations attempt to elucidate the pathogenesis of viral infections of the adult and fetus using immunological and virological techniques. Herpes simplex virus, CMV, and varicella have been agents which have received particular attention in these studies, since they have significant neurotropic capabilities in terms of newborn and adult encephalitides. There is considerable speculation that these viruses may have latent "slow" virus potential in relationship to chronic diseases of humans, including carcinoma and central nervous system infection. Investigation of the clinical and biological properties of the two strains of herpes simplex virus have permitted more definitive establishment of such capabilities. Furthermore, the therapeutic potential of experimental drugs in animals with these infections may prove useful for future treatment of humans.

Proposed Course of the Project: Antibody complement mediated cytotoxicity studies with serum from carcinoma in situ patients will be studied. A study of PML, SV₄₀ in owl monkeys is being done to determine if PML can be

produced in experimental animals. The carp agent will be examined for possible relationship to MS. An infectious mononuclear antigen will be produced for possible diagnostic test using direct hemagglutination test.

Publications:

Thong, Y.H., Vincent, M.M., Hensen, S.A., Fuccillo, D.A., Rola-Pleszczynski, M., and Bellanti, J.A.: Depressed Specific Cell-Mediated Immunity to Herpes Simplex Virus Type 1 in Patients with Recurrent Herpes Labialis. Infection and Immunity, p. 76-80, July 1975.

Palmer, A.E., London, W.T., Nahmias, A.J., Naib, Z.M., Tunca, J., Fuccillo, D.A., Ellenberg, J.H. and Sever, J.L.: A Preliminary Report on Investigation of Oncogenic Potential of Herpes Simplex Virus Type 2 in Cebus Monkeys. Cancer Research, Vol. 36, p. 807-809, Feb. 1976.

Oldstone, M.B., Bokisch, V.A., Dixon, F.J., Barbosa, L.H., Fuccillo, D.A., and Sever, J.L.: Subacute Sclerosing Panencephalitis: Destruction of Human Brain Cells by Antibody and Complement in an Autologous System, Clin. Immuno. Immunopath, 4, p. 52-58, 1975.

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| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | | U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | | PROJECT NUMBER Z01 NS 01984-05 ID | |
| PERIOD COVERED: July 1, 1975 through June 30, 1976 | | | | | |
| TITLE OF PROJECT (80 characters or less) Maternal Infection and Pregnancy Outcome | | | | | |
| NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT | | | | | |
| PI: | | Dr. David A. Fuccillo | | Head | |
| | | Dr. John L. Sever | | Chief | |
| | | | | Section on Neurovirology IDB, IRP, NINCDS Infectious Diseases Branch IRP, NINCDS | |
| Others: | | Dr. William T. London | | Head | |
| | | Mrs. Renee Traub | | Microbiologist | |
| | | | | Section on Experimental Pathology, IDB, IRP, NINCDS Section on Neurovirology IDB, IRP, NINCDS | |
| COOPERATING UNITS (if any) Pennsylvania Hospital University of Tennessee University of Oklahoma | | | | | |
| LAB/BRANCH Infectious Diseases Branch | | | | | |
| SECTION Neurovirology | | | | | |
| INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20014 | | | | | |
| TOTAL MANYEARS: | | PROFESSIONAL: | | OTHER: | |
| 4.5 | | 1.0 | | 3.5 | |
| SUMMARY OF WORK (200 words or less - underline keywords) Influenza virus, when inoculated in utero in baby monkeys produced <u>hydrocephalus</u> at birth. A middle class <u>population</u> isolation rates for CMV from mothers cervixes and babies urine were found to be 1.2% and .2% respectively. <u>Maternal infection</u> for herpes virus isolation was .09% and .3% for newborn babies. No significant difference in antibody titers for Coxsackie B virus could be found in neonatal diabetes. | | | | | |

Project Description:

Objectives: Of the 3.5 million children born each year in the U.S., approximately 3% are mentally retarded. It appears that as many as 10% of these cases may be attributed to infectious disease. Our objective is to utilize various virological techniques in an intensive study of viruses to determine their role in the production of birth defects and related abnormalities. To develop virological techniques necessary for the investigation of the natural course of the disease as caused by the infectious agents.

Methods Employed: New virus isolation techniques and serum neutralization tests are used for large scale testing in the study of pregnant women and their children. The development of new techniques, enzyme linked antibodies studies and radioimmune assay tests are being perfected. These tests are being used to establish the reliability of other tests and to determine the frequency of antibody changes during pregnancy and the effect of these infections on the developing embryo. Babies born with high IgM are being cultured and serological studies done to determine if fetal infection occurred. Neonatal deaths and spontaneous abortions were studied to determine abnormal outcomes.

Animal studies with various viruses inoculated in utero will be studied for their teratogenic affects.

Pregnant women will be studied for genital infection with herpes virus and cytomegalovirus. The affects of virus on pregnancy during gestational period will be studied. Amniotic fluid will be taken during pregnancy to determine presence of virus, bacterial of virus and abnormal levels of globulins. Follow up on patients with herpes will be done for abnormal Pap smears and cancer of the cervix. Influenza viruses, when inoculated in utero in baby monkeys, produce hydrocephalus at birth.

Major Findings: Naval Hospital isolation rates for CMV from mothers cervixes and babies urine were found to be 1.2% and .2% respectively. Rates for herpes was .09% for mothers and .3% for babies. The Philadelphia viral isolation study on pregnant women were CMV .78% and Herpes .65%. No increased IgM were found in any of the amniotic fluids studied. A number of viral isolations were made from the high IgM study and study summarized. Coxsackie B neutralization studies on neonatal diabetes have shown that this virus is probably not involved with this disease.

Significance of the Program to the Institute: The results from these studies help determine what affect virus infection has on abnormal pregnancy outcomes and also provide valuable information on the epidemiological aspects of virus infections. The relationship of these in utero infections to various degrees of neurological dysfunction in later years of life is still unknown.

Proposed Course of the Project: Mumps and reoviruses will be inoculated in monkeys to observe for production of tetatogenic affects. Studies are still in progress on cytomegalovirus infections during pregnancy and in several population groups. Additional studies are being conducted on Herpes Type I and II infections in women. Drug evaluation of Phosphon-acetic acid for CMV and Herpes hominis is being conducted in monkeys. Other experimental drugs are also being tested. Approximately 16,000 sera from abnormal pregnancy outcomes are being tested for Herpes I and II, CMV and Toxoplasma.

Publications:

Stagno, S., Reynolds, D., Tsiantos, A., Fuccillo, D.A., Smith, R., Tiller, M., and Alford, C.A.: Cervical Cytomegalovirus Excretion in Pregnant and Nonpregnant Women: Suppression in Early Gestation. Journal of Infectious Diseases, Vol. 131, No. 5, pp. 522-527, May 1975.

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| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 NS 01985-05 ID | | | | | | | | | | | | | | | | | | |
| PERIOD COVERED: July 1, 1975 to June 30, 1976 | | | | | | | | | | | | | | | | | | | | |
| TITLE OF PROJECT (80 characters or less) Presence of Viral Antigens or Antibodies in Perinatal and Neurological Diseases. | | | | | | | | | | | | | | | | | | | | |
| NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT | | | | | | | | | | | | | | | | | | | | |
| <table style="width: 100%; border: none;"> <tr> <td style="width: 35%;">PI: Dr. David L. Madden</td> <td style="width: 35%;">Head, Unit on Microbiology</td> <td style="width: 30%;">IDB, IRP, NINCDS</td> </tr> <tr> <td colspan="3"> </td> </tr> <tr> <td>Other: Dr. David Fuccillo</td> <td>Head, Sec. on Neurovirology</td> <td>IDB, IRP, NINCDS</td> </tr> <tr> <td>Mrs. Mary Krasny</td> <td>Microbiologist</td> <td>IDB, IRP, NINCDS</td> </tr> <tr> <td>Mrs. Aurella Krezlewicz</td> <td>Microbiologist</td> <td>IDB, IRP, NINCDS</td> </tr> <tr> <td>Dr. William London</td> <td>Head, Sec. on Experimental Pathology</td> <td>IDB, IRP, NINCDS</td> </tr> </table> | | | PI: Dr. David L. Madden | Head, Unit on Microbiology | IDB, IRP, NINCDS | | | | Other: Dr. David Fuccillo | Head, Sec. on Neurovirology | IDB, IRP, NINCDS | Mrs. Mary Krasny | Microbiologist | IDB, IRP, NINCDS | Mrs. Aurella Krezlewicz | Microbiologist | IDB, IRP, NINCDS | Dr. William London | Head, Sec. on Experimental Pathology | IDB, IRP, NINCDS |
| PI: Dr. David L. Madden | Head, Unit on Microbiology | IDB, IRP, NINCDS | | | | | | | | | | | | | | | | | | |
| | | | | | | | | | | | | | | | | | | | | |
| Other: Dr. David Fuccillo | Head, Sec. on Neurovirology | IDB, IRP, NINCDS | | | | | | | | | | | | | | | | | | |
| Mrs. Mary Krasny | Microbiologist | IDB, IRP, NINCDS | | | | | | | | | | | | | | | | | | |
| Mrs. Aurella Krezlewicz | Microbiologist | IDB, IRP, NINCDS | | | | | | | | | | | | | | | | | | |
| Dr. William London | Head, Sec. on Experimental Pathology | IDB, IRP, NINCDS | | | | | | | | | | | | | | | | | | |
| COOPERATING UNITS (if any) Lynchburg Training School and Hospital Electronucleonics, Inc. Microbiological Associates, Inc. | | | | | | | | | | | | | | | | | | | | |
| LAB/BRANCH Infectious Diseases Branch | | | | | | | | | | | | | | | | | | | | |
| SECTION Neurovirology | | | | | | | | | | | | | | | | | | | | |
| INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Md. 20014 | | | | | | | | | | | | | | | | | | | | |
| TOTAL MANYEARS: 2.25 | PROFESSIONAL: 0.75 | OTHER: 1.5 | | | | | | | | | | | | | | | | | | |
| SUMMARY OF WORK (200 words or less - underline keywords) Efforts have been made to determine the etiological agent in <u>multiple sclerosis</u> . Several new techniques to determine <u>humoral</u> and <u>cellular immune responses</u> have been made. These include <u>direct migration inhibition</u> , <u>lymphocyte cytotoxicity</u> and <u>comediated antibody cytotoxicity studies</u> . Mice have been inoculated with human MS and control brain material in an effort to confirm the association of the <u>mouse PMN depressing agent</u> and multiple sclerosis. | | | | | | | | | | | | | | | | | | | | |
| A highly specific antibody against <u>Simian Hemorrhagic fever virus</u> has been produced in guinea pigs. Identification of chronic carriers using the complement fixation test were not very successful. | | | | | | | | | | | | | | | | | | | | |

Project Description:

Objectives: To isolate and identify viral antigens and/or antibodies. To utilize these antigens for more specific, rapid, sensitive identification of antibody and/or a more accurate identification of the viral agent in disease. To utilize these antibody for more specific, rapid, sensitive identification of antigen and/or a more accurate identification of the viral agent in disease. To define the humoral and cellular immune response of patients with neurological disease to these antigens. To determine the relationship between hepatitis A or B infection in pregnant women and mental retardation, congenital jaundice and postnatal jaundice.

Methods Employed: Human tissue culture lines chronically infected with SSPE measles virus, herpes virus type I and herpes virus type II cytomegalovirus (CMV) have been used to determine the immunological response of Subacute Sclerosing Panencephalitis (SSPE), Multiple Sclerosis (MS) patients and matched pal controls to these antigens. The infected cells have been labeled with 51 chromium. Target cells and various concentrations of lymphocytes or serum are reacted for 18 and 24 hours and the amount of 51 chromium specifically released is determined.

Simian hemorrhagic fever (SHF) an acute viral disease of rhesus monkeys and a chronic viral disease in other simian species has been studied. Lack of an in vitro diagnostic test has hampered studies of this disease. A highly specific SHF antibody has been produced by inoculating infectious rhesus monkey serum into guinea pigs. These animals are bled and normal rhesus serum antibodies are absorbed using normal rhesus monkey serum fixed in an acrylamide matrix leaving the specific SHF antibody. A complement fixation test using the specific SHF guinea pig antibody and infectious rhesus monkey serum has been developed. Antibody against the tissue culture adapted strain has been produced.

Major Findings: A technique for the rapid transfer for radioactive material from microtiter plates to the counting chamber has been developed. These tips have eliminated much of the nonspecific release encountered with other techniques.

Lymphocytes from SSPE patients responded to a SSPE strain of measles infected cells in a manner similar to lymphocytes from controls. A factor, which interfered with the expected activity of sensitized lymphocytes to measles virus was detected in some cerebral spinal fluid obtained from SSPE patients. The blocking activity varied from 100% in 4 of 20 SSPE patients to no blocking in 2 of the SSPE patients.

Lymphocytes from MS patients responded to a SSPE strain of measles infected cells in a manner similar to lymphocytes from controls. No blocking factor was found in serum of cerebral spinal fluid from MS patients.

A highly specific antibody against SHF has been produced in guinea pigs which reacts in a complement fixation test with antigen in blood of acutely ill rhesus monkeys and chronically infected Patas and African Green monkeys.

The tissue culture produced antigen does not react against this serum but one passage in the rhesus monkey resulted in the development of a reactive antigen. This indicates that the tissue culture adapted strain has lost some antigenic properties. At least, two strains of SHF virus exists, the LVR strain which grows well in tissue culture and the Corbell strain which cannot be propagated in tissue culture cells. Physical characterization of both strain indicated that they both have the same density 1.14. However by electron microscopic studies the Corbell strains seems to have few intact particles than the LVR strain.

Studies on the association of hepatitis in pregnant women and observable disease in newborns has been continued. Preliminary studies indicate that the etiology of Down's Syndrome is not associated with hepatitis A. In 700 serum samples obtained from the perinatal studies neither hepatitis B antigen or antibody detected in the serum of mothers with abnormal children at a higher incidence than mothers with normal children.

Significance of the Program to the Institute : The development of more specific antigens or antibodies which measure more accurately the immunological status of an individual is needed. Highly specific antigens or antibody may help identify the biological differences between nonpathogenic and pathogenic strains of these organisms and identify the etiology of obscure diseases. The studies on SHF will aid in determining the epizootiology of the disease and subsequent prevention. This will help preserve a dwindling supply of nonhuman primates.

Proposed Course of the Project: Further studies will be done to identify the antigens associated with the measles and rubella, Herpes I, Herpes II, and CMV, and SHF viruses. Cellular and humoral immunity studies are being expanded in an effort to detect small amounts of antigen on intact cells and immunological response differences which may account for disease. Efforts will be made to utilize the immune electron microscopic technique in detection of agents associated with multiple sclerosis and other neurological diseases of unknown etiology.

Publications: None

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| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER <div style="text-align: center; font-weight: bold;">Z01 NS 02036-04 ID</div> |
| PERIOD COVERED: <div style="text-align: center;">July 1, 1975 to June 30, 1976</div> | | |
| TITLE OF PROJECT (80 characters or less) <div style="text-align: center;">Immunology of Chronic Neurologic Infections</div> | | |
| NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT | | |
| PI: Dr. Maneth Gravell Dr. William London Dr. Monique Dubois-Dalcq Other: Mrs. Rebecca Hamilton Mr. Leonard Moore | Head, Unit on Neurovirology Diseases Head, Sec. on Experimental Pathology Head, Sec. on Electron Microscopy Biologist Biological Lab Technician | IDB, IRP, NINCDS IDB, IRP, NINCDS IDB, IRP, NINCDS IDB, IRP, NINCDS IDB, IRP, NINCDS |
| COOPERATING UNITS (if any) <div style="text-align: center;">None</div> | | |
| LAB/BRANCH <div style="text-align: center;">Infectious Diseases Branch</div> | | |
| SECTION <div style="text-align: center;">Neurovirology</div> | | |
| INSTITUTE AND LOCATION <div style="text-align: center;">NINCDS, NIH, Bethesda, Md. 20014</div> | | |
| TOTAL MANYEARS: <div style="text-align: center;">0.7</div> | PROFESSIONAL: <div style="text-align: center;">0.3</div> | OTHER: <div style="text-align: center;">0.4</div> |
| SUMMARY OF WORK (200 words or less - underline keywords) | | |
| <p>An African Green Monkey has been inoculated with the "BIKEN" strain of defective measles virus to establish an animal model for human subacute sclerosing panencephalitis (SSPE). <u>Insufficient time has elapsed since this project was initiated to draw firm conclusions.</u> If a suitable subhuman primate model of SSPE results from this work, it will be used to study the role of antibody and/or cellular immunity in the development of chronic measles infection and to develop improved regimens for the control and treatment of human SSPE.</p> | | |

Project Description:

Objectives: To establish a model of human subacute sclerosing panencephalitis (SSPE) in subhuman primates. This model is to be used to determine the role of humoral and/or cellular immunity in the development of chronic measles infection and to evaluate various chemo- and/or immunotherapeutic reagents for the treatment of SSPE.

Methods Employed: A measles antibody negative African green monkey has been inoculated with cells infected with the "BIKEN" strain of measles virus. The inoculated animal is being observed for neurological and/or other clinical symptoms of disease. If a predictable animal model of SSPE can be established, it will be used to monitor the development of antibody and cellular immunity to measles virus and to develop improved methods of control and treatment of SSPE.

Major Findings: Japanese investigators recently reported that the "BIKEN" strain of defective measles virus will produce an SSPE-like disease in the African green monkey. We have inoculated an African green monkey with "BIKEN" strain measles virus to verify this report. First signs of SSPE-like disease were reported to occur approximately 2 months after inoculation with "BIKEN" strain virus. Our study has been in effect for only one month and no signs of acute measles infection were observed during this period.

Significance of the Program to the Institute: No suitable animal model of human SSPE is currently available. The development of an animal model would make it possible to study the role of the immune system in the development of SSPE-like disease and to study various regimens which might be useful in prevention and/or therapy of this disease.

Proposed Course of the Project : During the first year major emphasis will be placed on establishing a reliable subhuman primate model of SSPE and to perfecting assays which accurately monitor the humoral and/or cellular immune responses of inoculated animals to measles virus infection. If the first stage of the project is successful, various treatment regimens will be studied.

Publications: None

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| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | | U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 NS 02069-03 ID | | | | | | | | | | | | |
| PERIOD COVERED July 1, 1975 to June 30, 1976 | | | | | | | | | | | | | | | |
| TITLE OF PROJECT (80 characters or less) Cell Mediated Immunity and SSPE | | | | | | | | | | | | | | | |
| NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT <table border="0"> <tr> <td>PI: Dr. Maneth Gravell</td> <td>Head, Unit on Neurovirology</td> <td>IDB, IRP, NINCDS</td> </tr> <tr> <td></td> <td>Diseases</td> <td></td> </tr> <tr> <td>Other: Mrs. Rebecca Hamilton</td> <td>Biologist</td> <td>IDB, IRP, NINCDS</td> </tr> <tr> <td>Dr. Michael Blaese</td> <td>Microbiologist</td> <td>NCI</td> </tr> </table> | | | | PI: Dr. Maneth Gravell | Head, Unit on Neurovirology | IDB, IRP, NINCDS | | Diseases | | Other: Mrs. Rebecca Hamilton | Biologist | IDB, IRP, NINCDS | Dr. Michael Blaese | Microbiologist | NCI |
| PI: Dr. Maneth Gravell | Head, Unit on Neurovirology | IDB, IRP, NINCDS | | | | | | | | | | | | | |
| | Diseases | | | | | | | | | | | | | | |
| Other: Mrs. Rebecca Hamilton | Biologist | IDB, IRP, NINCDS | | | | | | | | | | | | | |
| Dr. Michael Blaese | Microbiologist | NCI | | | | | | | | | | | | | |
| COOPERATING UNITS (if any) None | | | | | | | | | | | | | | | |
| LAB/BRANCH Infectious Diseases Branch | | | | | | | | | | | | | | | |
| SECTION Neurovirology | | | | | | | | | | | | | | | |
| INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Md. 20014 | | | | | | | | | | | | | | | |
| TOTAL MANYEARS: 0.6 | PROFESSIONAL: 0.2 | OTHER: 0.4 | | | | | | | | | | | | | |
| SUMMARY OF WORK (200 words or less - underline keywords) The Vr/60 strain of measles virus produces a characteristic cytopathology in vero cells but infectious cell-free virus is not formed. When Vr/60 chronically infected cells were incubated with lymphocytes from normal donors or SSPE patients a marked decrease in plaque formation was noted. <u>Lymphocyte</u> from individuals with immune deficiencies (a gammalobulinemia, Sezzari syndrome and IgA deficiency) failed to reduce plaque counts. It has been suggested that this test may be a measure of measlesvirus-specific cellular immunity. If this is true, it can be concluded that SSPE patients have normal cellular immunity to measles virus. However, this conclusion cannot be drawn unequivocally because the mechanism of <u>plaque reduction</u> in this <u>test</u> has not been elucidated. | | | | | | | | | | | | | | | |

Project Description:

Objective: To develop an in vitro test to evaluate cellular immunity of subacute sclerosing panencephalitis patients to measles virus.

Methods Employed: The Vr/60 cell line chronically infected with measles virus produces a characteristic CPE consisting of small plaques when co-cultivated with Vero cells. Cell-free virus has not been obtained from these cultures, however, the cytopathic agent has been shown clearly to be measles virus.

Lymphocytes from SSPE patients and normal controls will be obtained by the Ficoll-Isopaque gradient method.

The Vr/60 target cells are mixed with lymphocyte suspensions and incubated for 24 hours. After this incubation, lymphocytes are removed and cultures refed with fresh medium. All cultures are fixed and stained when control Vr/60 cells show typical plaques. The test is evaluated based on plaque reduction compared to controls.

Major Findings: Lymphocytes from 25 normal donors and 20 SSPE patients were studied in the plaque assay described. Comparable plaque reduction was produced by lymphocytes from SSPE patients and normal controls. However, lymphocytes from individuals with immune deficiencies (agammaglobulinemia, Sezzari syndrome and IgA deficiency) failed to reduce plaque counts. These results suggested that SSPE patients have normal cellular immunity to measles virus. This conclusion, can not be drawn unequivocally until the mechanism of plaque reduction is more completely understood. Future work will be directed to this end.

Significance to the Program of the Institute: To date no reliable test for measuring measles virus-specific cellular immunity exists. Recent findings suggest that the cellular immunity of SSPE patients to measles virus may be impaired. Development of a reliable test to determine cellular immunity to measles virus may be of value in understanding the pathogenesis of SSPE and aid in the development of new approaches to the prevention and treatment of the disease.

Proposed Course of the Project: Future work will be directed to understanding the mechanism of reduction of measles virus plaques. Additional patients with various immune deficiency diseases will be studied to pinpoint the criteria required to obtain plaque reduction.

Publications: None

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| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | | U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | | PROJECT NUMBER Z01 NS 02070-03 ID | |
| PERIOD COVERED July 1, 1975 through June 30, 1976 | | | | | |
| TITLE OF PROJECT (80 characters or less) Separation Techniques Involving Globulin Proteins in Cerebrospinal Fluid | | | | | |
| NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT | | | | | |
| PI: | | Dr. Richard S. Johannes | | Research Associate IDB, IRP, NINCDS | |
| Others: | | Mr. Otto Gutenson | | Biologist IDB, IRP, NINCDS | |
| COOPERATING UNITS (if any) None | | | | | |
| LAB/BRANCH Infectious Diseases Branch | | | | | |
| SECTION Neurovirology | | | | | |
| INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20014 | | | | | |
| TOTAL MANYEARS: 0 | | PROFESSIONAL: 0 | | OTHER: 0 | |
| SUMMARY OF WORK (200 words or less - underline keywords) Project terminated due to detachment of Dr. Johannes. | | | | | |

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| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 NS 02071-03 ID |
| PERIOD COVERED July 1, 1975 through June 30, 1976 | | |
| TITLE OF PROJECT (80 characters or less) Cerebrospinal Fluid Electrophoresis-For Diagnosis and Prognosis of MS and SSPE | | |
| NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT <div style="display: flex; justify-content: space-between; margin-top: 10px;"> <div style="width: 30%;"> PI: Dr. Richard S. Johannes </div> <div style="width: 65%; text-align: right;"> Research Associate, IDB, IRP, NINCDS </div> </div> | | |
| COOPERATING UNITS (if any) None. | | |
| LAB/BRANCH Infectious Diseases Branch | | |
| SECTION Neurovirology | | |
| INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20014 | | |
| TOTAL MANYEARS: <div style="text-align: center;">0</div> | PROFESSIONAL: <div style="text-align: center;">0</div> | OTHER: <div style="text-align: center;">0</div> |
| SUMMARY OF WORK (200 words or less - underline keywords) Project terminated due to detachment of Dr. Johannes. | | |

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| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 NS 02130-02 ID |
| PERIOD COVERED: July 1, 1975 through June 30, 1976 | | |
| TITLE OF PROJECT (80 characters or less) Induction of Cellular Fusion by the Use of Cell Free Systems and Its Relation to Virus Rescue | | |
| NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT PI: Dr. Richard S. Johannes Research Associate IDB, IRP, NINCDS Others: Mr. Otto Gutenson Biologist IDB, IRP, NINCDS | | |
| COOPERATING UNITS (if any) None | | |
| LAB/BRANCH Infectious Diseases Branch | | |
| SECTION Neurovirology | | |
| INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20014 | | |
| TOTAL MANYEARS: 0 | PROFESSIONAL: 0 | OTHER: 0 |
| SUMMARY OF WORK (200 words or less - underline keywords) Project terminated due to detachment of Dr. Johannes. | | |

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| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 NS 02131-02 ID |
| PERIOD COVERED July 1, 1975 through June 30, 1976 | | |
| TITLE OF PROJECT (80 characters or less) Determination of Antigenicity of DNA Viral Proteins for Vaccine Use. | | |
| NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT PI Dr. Michael T. Murphy Microbiologist IDB, IRP, NINCDS | | |
| COOPERATING UNITS (if any) None | | |
| LAB/BRANCH Infectious Diseases Branch | | |
| SECTION Neurovirology | | |
| INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20014 | | |
| TOTAL MANYEARS: .1 | PROFESSIONAL: .1 | OTHER: |
| SUMMARY OF WORK (200 words or less - underline keywords) To obtain <u>viral</u> proteins which are nucleic acid free from <u>DNA</u> viruses, particularly those thought to be associated with neurological diseases. To develop an animal test system to evaluate the <u>antigenicity</u> of these proteins for possible <u>vaccine</u> usefulness. Due to the negative results of initial experiments, it was concluded that this project be terminated. | | |

Project Description:

Objectives: To obtain viral proteins which are nucleic acid free from DNA viruses, particularly those thought to be associated with neurological diseases. To develop an animal test system to evaluate the antigenicity of these proteins for possible vaccine usefulness.

Methods Employed: Proteins of viral origin obtained from infection of tissue cultures will be extracted by several means, e.g. sonication, treatment with detergents, and osmotic disruption. Purification of the viral proteins will be done with classical enzymological methods, in addition to the purification by isoelectric focusing. The latter methodology will achieve the required purification without loss of biological function. These proteins will be injected into model animal systems for evaluation of antigenicity.

Major Findings: Several attempts to isolate glycoproteins of the Herpes I and II viruses resulted in extremely low concentrations of antigen (protein). This material was injected into model guinea pig systems and the results were inconclusive or negative with regard to production of antibody.

Significance of the Program to the Institute: This study represents an initial effort into the field of prevention of possible neurological diseases, as opposed to treatment after the fact. It is hoped that information gained in these antigenicity studies will be correlated to other diseases being studied by this institute.

Proposed Course of the Project: Due to the negative results of initial experiments, it was concluded that this project be terminated.

Publications: None

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| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 NS 02132-02 ID |
| PERIOD COVERED: July 1, 1975 through June 30, 1976 | | |
| TITLE OF PROJECT (80 characters or less) Characterization of SSPE-Like Particles in Latent and Lytic Tissue Culture Conditions. | | |
| NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT | | |
| PI: | Dr. Michael F. Murphy | Microbiologist IDB, IRP, NINCDS |
| Others: | Dr. Morag Ferguson Otto Gutenson | Visiting Fellow IDB, IRP, NINCDS Biologist IDB, IRP, NINCDS |
| | | |
| COOPERATING UNITS (if any) None | | |
| LAB/BRANCH Infectious Diseases Branch | | |
| SECTION Neurovirology | | |
| INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20014 | | |
| TOTAL MANYEARS: | PROFESSIONAL: | OTHER: |
| 2.2 | 1.6 | .6 |
| SUMMARY OF WORK (200 words or less - underline keywords) To study and identify changes in <u>structural proteins</u> and other biochemical differences occurring in the transformation of a lytic viral infection to the latent or chronic state where a viral genome or subunit resides inside a cell and causes no observable cytotoxicity. To ascertain the <u>mode of replication</u> of these two different states of infection. | | |

Project Description:

Objectives: To study and identify changes in structural proteins and other biochemical differences occurring in the transformation of a lytic viral infection to the latent or chronic state where a viral genome or subunit resides inside a cell and causes no observable cytotoxicity. To ascertain the mode of replication of these two different states of infection.

Methods Employed: Nucleic acids which have been radio-labeled will be extracted from both types of infections and compared. The sizes of these genomes of apticles will be obtained through gradient centrifugation and agarose chromatography. Homology between species will be examined by nucleic acid hybridization. Viral proteins will be separated and sized by polyacrylamide electrophoresis and electrofocusing. Determination of protein functions will also be studied, e.g. RNA dependent RNA polymerase.

Major Findings: The chronically infected 72046 cell line contains a distinct morphological type of particle which upon freezing and thawing is infectious. Infectious particles of Halle virus and Edmonston virus appear to contain at least six comparable viral proteins. Prolonged undiluted passage of plaque purified virus of both SSPE (Halle), and measles reveals properties attributable to the production of a defective interfering particle. Nucleic acid studies have been extremely difficult due to the instability of the extracted viral RNA. More techniques of isolation are being examined.

Significance of the Program to the Institute: This study will give information into the complexities of chronic or latent infections of which neurological diseases are thought to be a part. Identification of differences between lytic and rhonic infection may be applied in the search for other agents causing neurological disease.

Proposed Course of the Project: Many of the lytic viruses appear to propagate defective particles which interfere with replication of the infective genome. Many viruses associated with diseases have been shown to be capable of entering a latent state in the host cell. It is proposed that this initial investigation be extended to include studies on CMV, Herpes, and other viruses in this category.

Publications:

Dubois-Dalcq, M., Reese, T.S., Murphy, M., and Fuccillo, D.: Defective Bud Formation in Human Cells Chronically Infected with SSPE virus. Virology, 1975. (In Press).

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| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 NS 02133-02 -ID | | | | | | | | |
| PERIOD COVERED July 1, 1975 through June 30, 1976 | | | | | | | | | | |
| TITLE OF PROJECT (80 characters or less) Enzymology As a Diagnostic Probe for Herpes and other DNA Viruses. | | | | | | | | | | |
| NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT <table style="width: 100%; border: none;"> <tr> <td style="width: 15%;">PI:</td> <td style="width: 35%;">Dr. Michael F. Murphy</td> <td style="width: 30%;">Microbiologist</td> <td style="width: 20%;">IDB,IRP, NINCDS</td> </tr> <tr> <td>Others:</td> <td>Mr. Otto Gutenson</td> <td>Biologist</td> <td>IDB, IRP, NINCDS</td> </tr> </table> | | | PI: | Dr. Michael F. Murphy | Microbiologist | IDB,IRP, NINCDS | Others: | Mr. Otto Gutenson | Biologist | IDB, IRP, NINCDS |
| PI: | Dr. Michael F. Murphy | Microbiologist | IDB,IRP, NINCDS | | | | | | | |
| Others: | Mr. Otto Gutenson | Biologist | IDB, IRP, NINCDS | | | | | | | |
| COOPERATING UNITS (if any) None | | | | | | | | | | |
| LAB/BRANCH Infectious Diseases Branch | | | | | | | | | | |
| SECTION Neurovirology | | | | | | | | | | |
| INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20014 | | | | | | | | | | |
| TOTAL MANYEARS: .2 | PROFESSIONAL: .1 | OTHER: .1 | | | | | | | | |
| SUMMARY OF WORK (200 words or less - underline keywords) To develop an assay of <u>thymidine kinase activity</u> to detect the possible presence of <u>Herpes virus</u> in certain diseases. To examine the possibility to extend these studies to <u>other DNA viruses</u> . | | | | | | | | | | |

Project Description:

Objectives: To develop an assay of thymidine kinase activity to detect the possible presence of Herpes virus in certain diseases. To examine the possibility to extend these studies to other DNA viruses.

Methods Employed: Cytoplasm of control cells and chronically infected cells thought to contain Herpes viral genome are the source for the enzyme. An assay method for the enzyme is now being investigated. Polyacrylamide gel electrophoresis will be used to separate the different activities which have been shown to have different electrophoretic mobilities. This method will then be applied to examine CSF and serum of patients with neurological disease.

Major Findings: Under present conditions, Herpes thymidine kinase is very labile. New published data has revealed that the system works well. Our system using BHK-21 cells worked for Herpes I. Extension of these studies to CMV has been hampered by difficulty in growing titers high enough to infect at multiplicities needed for synthesis of the enzyme. Chronically infected cell lines must now be tested.

Significance of the Program to the Institute: This study is directed toward early identification of neurological disease associated with viral antigens. Further, detection of antigens of DNA viruses known to be associated with some CNS diseases would alert the medical practitioner to the possibility that babies of infected mothers should be closely examined and followed.

Proposed Course of the Project: To investigate the presence of either the entire genome or partial fragments of viruses which have been thought to be associated with neurological diseases, a detection assay of the specifically induced viral thymidine kinase activity will be used. The results of the assay system will be correlated to other diagnostic criteria such as virus isolation and fluorescent antibody tests.

Publications: None

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| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 NS 02134-02 ID |
| PERIOD COVERED July 1, 1975 through June 30, 1976 | | |
| TITLE OF PROJECT (80 characters or less) Biochemical Effects of Antiviral Agents on Infected Cells and Control Cells. | | |
| NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT | | |
| PI: | Dr. Michael T. Murphy | Microbiologist IDB, IRP, NINCDS |
| Others: | Mr. Otto Gutenson | Biologist IDB, IRP, NINCDS |
| COOPERATING UNITS (if any) None | | |
| LAB/BRANCH Infectious Diseases Branch | | |
| SECTION Neurovirology | | |
| INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20014 | | |
| TOTAL MANYEARS: .5 | PROFESSIONAL: .2 | OTHER: .3 |
| SUMMARY OF WORK (200 words or less - underline keywords) | | |
| <p>To examine the biochemical effects exhibited by the <u>antiviral, ribavirin</u>, against control and infected cells in tissue culture. To compare and evaluate this <u>synthetic nucleotide</u> as an antiviral agent to be used against <u>slow viruses</u> containing either RNA or DNA.</p> | | |

Project Description:

Objectives: To examine the biochemical effects exhibited by the antiviral, ribavirin, against control and infected cells in tissue culture. To compare and evaluate this synthetic nucleotide as an antiviral agent to be used against slow viruses containing either RNA or DNA.

Methods Employed: Tissue cultures of control Vero cells and Vero cells infected with Halle (SSPE) as well as two chronic SSPE infected cell lines were exposed to ribavirin to determine effects on macromolecular synthesis and production of infectious particles. Plaque reduction and incorporation of radio-labeled compounds will be examined to determine the effectiveness of this antiviral agent in treatment of measles and/or SSPE diseases.

Major Findings: Both chronically infected cell lines display slightly different patterns from the control cells. However, proteins synthesis is not effected due to the production of viral antigen as detected by fluorescent antibody staining technique. The antiviral effectively reduces the plaques from chronic infections as well as from lytic infections of Halle (SSPE) virus in Vero cells.

Significance of the Program to the Institute: Any broad spectrum antiviral which is not toxic to the patient at effective dosage is important to any program. Indeed this antiviral may be tailored to neurological diseases in view of the differing results obtained between infected and non-infected cells.

Proposed Course of the Project: To investigate the effects of different levels of the antiviral and their efficacy. Also the examination of other viral agents, such as DNA virus will be examined and compared to SSPE.

Publications: None

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|--|---|--|-----|-----------------|-------------------------------|--|-----------------|-----------------------------------|--|----------------|--------------------|--|-------------------|--------------------------------|--|---------------|----------------------------|--------|---------------|-----------------------------------|--|-------------------|------------------------------------|--|----------------|---------------------------------------|--|-----------------|---|
| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 NS 00972-05 ID | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| PERIOD COVERED <u>July 1, 1975 to June 30, 1976</u> | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| TITLE OF PROJECT (80 characters or less) <u>Role of Viruses and Other Micro-Organisms in the Perinatal Period of Experimental Animals.</u> | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT <table style="width: 100%; border: none;"> <tr> <td style="width: 10%; vertical-align: top;">PI:</td> <td style="width: 40%;">Dr. W.T. London</td> <td style="width: 50%;">Head, Exp. Path., IDB, NINCDS</td> </tr> <tr> <td></td> <td>Dr. A.E. Palmer</td> <td>Res. Vet. Exp. Path., IDB, NINCDS</td> </tr> <tr> <td></td> <td>Dr. J.L. Sever</td> <td>Chief, IDB, NINCDS</td> </tr> <tr> <td></td> <td>Dr. D.A. Fuccillo</td> <td>Head, Neurovirol., IDB, NINCDS</td> </tr> <tr> <td></td> <td>Dr. J.M. Rice</td> <td>Senior Scientist, Exp. NCI</td> </tr> <tr> <td style="vertical-align: top;">Other:</td> <td>Mrs. A.C. Ley</td> <td>Microbiol., Immunol., IDB, NINCDS</td> </tr> <tr> <td></td> <td>Mrs. B.L. Curfman</td> <td>Biologist, Exp. Path., IDB, NINCDS</td> </tr> <tr> <td></td> <td>Mr. R.L. Brown</td> <td>Bio.Lab.Tech, Exp. Path., IDB, NINCDS</td> </tr> <tr> <td></td> <td>Mrs. G.M. Brown</td> <td>Bio. Lab.Tech., Exp. Path., IDB, NINCDS</td> </tr> </table> | | | PI: | Dr. W.T. London | Head, Exp. Path., IDB, NINCDS | | Dr. A.E. Palmer | Res. Vet. Exp. Path., IDB, NINCDS | | Dr. J.L. Sever | Chief, IDB, NINCDS | | Dr. D.A. Fuccillo | Head, Neurovirol., IDB, NINCDS | | Dr. J.M. Rice | Senior Scientist, Exp. NCI | Other: | Mrs. A.C. Ley | Microbiol., Immunol., IDB, NINCDS | | Mrs. B.L. Curfman | Biologist, Exp. Path., IDB, NINCDS | | Mr. R.L. Brown | Bio.Lab.Tech, Exp. Path., IDB, NINCDS | | Mrs. G.M. Brown | Bio. Lab.Tech., Exp. Path., IDB, NINCDS |
| PI: | Dr. W.T. London | Head, Exp. Path., IDB, NINCDS | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| | Dr. A.E. Palmer | Res. Vet. Exp. Path., IDB, NINCDS | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| | Dr. J.L. Sever | Chief, IDB, NINCDS | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| | Dr. D.A. Fuccillo | Head, Neurovirol., IDB, NINCDS | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| | Dr. J.M. Rice | Senior Scientist, Exp. NCI | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Other: | Mrs. A.C. Ley | Microbiol., Immunol., IDB, NINCDS | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| | Mrs. B.L. Curfman | Biologist, Exp. Path., IDB, NINCDS | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| | Mr. R.L. Brown | Bio.Lab.Tech, Exp. Path., IDB, NINCDS | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| | Mrs. G.M. Brown | Bio. Lab.Tech., Exp. Path., IDB, NINCDS | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| COOPERATING UNITS (if any) <u>George Washington University Medical School, Washington, D.C.</u> <u>U.S. Army Medical Research Institute of Infectious Diseases, Ft. Detrick, Frederick, Maryland</u> <u>Meloy Laboratories, Springfield, Virginia</u> | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| LAB/BRANCH <u>Infectious Diseases Branch</u> | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| SECTION <u>Section on Experimental Pathology</u> | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| INSTITUTE AND LOCATION <u>NINCDS, NIH, Bethesda, Maryland, 20014</u> | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| TOTAL MANYEARS: <u>4.5</u> | PROFESSIONAL: <u>2.5</u> | OTHER: <u>2.0</u> | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| SUMMARY OF WORK (200 words or less - underline keywords) <p>The <u>role of viruses</u> or <u>viral vaccines</u> as possible teratogens is under investigation. Several <u>arbovirus</u> vaccines produced for <u>human</u> or <u>animal</u> use have been found to cause fetal death or severe central nervous system anomalies when inoculated intracerebrally into 100 day old <u>Rhesus monkey fetuses</u>.</p> <p>None of the control animals used in the studies developed malformations. Additional studies are in progress in which the <u>pregnant animal</u> rather than the fetus is infected much earlier in gestation.</p> <p>Project Number 02037-04 has been incorporated into Project Number NS 00972-05 ID, shown above.</p> | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |

Project Description:

Objectives: To study the role of viruses and other microorganisms in the perinatal period, the infection of gravid and nongravid animals of several different species by parenteral routes with various viruses and other microorganisms to determine the effects of these agents on the animals and their fetal tissues.

Attempt to recover inoculated agents from the various animals and fetal tissues and the correlation of these reisolations with gestational age at inoculation and dosage given. Relate these findings with gross and histopathological findings. Correlate all of this information with serological findings.

Methods Employed: An investigation of the role of viruses and other microorganisms in the perinatal period by the continual use of experimental animals, tissue culture techniques, histopathological studies, and serological testing. Pregnant monkeys were inoculated by various routes with viruses and other microorganisms, and held in isolation chambers throughout the experiment. The animals were observed and monitored by serum samples, liver biopsy, spinal fluid and throat swabs for evidence of disease and/or effects on fetal tissue. Pregnant animals were delivered by cesarean section so all products of conception could be saved.

Isolation of virus from the specimens was done using co-cultivation techniques on tissue cultures. Fluorescent microscopy was performed to locate the viral antigen in the fetal tissues.

Major Findings: Several arbovirus vaccines produced for human or animal use have been found to cause fetal death or severe central nervous system anomalies when inoculated intracerebrally into 100 day old Rhesus monkey fetuses.

The vaccine strains of Dengue and yellow fever were so virulent that they invariably resulted in fetal death.

Venezuelan Equine Encephalitis (VEE) and Western Equine Encephalitis (WEE) vaccines produced microencephaly, porencephaly and hydrocephalus in all of the infected fetuses. The VEE vaccine invariably produced bilateral cataracts in inoculated animals.

The control animals in all groups did not develop antibody titers or anomalies.

Significance of the Program to the Institute: Research for animal models for human diseases known or suspected to cause malformations of the central nervous system should provide an insight into the pathogenesis of these anomalies. Epidemiological studies have shown that there are several viral teratogens in the human population. These could be more thoroughly studied

in animal models. Environmental agents alone or in combination with infectious agents may play a role in the development of certain types of congenital malformations. Animal models would certainly be useful in the study of these conditions.

Proposed Course of the Project: We have shown that several viral agents are are teratogenic in the fetal Rhesus monkey. To date, inoculations have been into the CNS of the fetus. Studies are now in progress where the mother is infected with the viral agent to determine if the virus can cross the placenta, infect the fetus and produce anomalies. These studies will be done in pregnant animals earlier in gestation (40 days instead of 100 days). Pregnant animals will be given a known neurogenic chemical carcinogen, ethylnitrosourea (ENU) several times during gestation. Concurrently, the fetus will be infected with Influenza A virus to determine if the combination is more teratogenic than either agent alone.

Publications:

London, W.T., Fuccillo, D.A., Sever, J.L., Kent, S.G.: Influenza Virus as a Teratogen in Rhesus Monkeys. Nature. 255: 483-484, 1975.

London, W.T., Levitt, N.H., Kent, S.G., Wong, V.C., and Sever, J.L.: Congenital Cerebral and Ocular Malformations Induced in Rhesus Monkeys by Venezuelan Equine Encephalitis Virus. Teratology. (In Press.)

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| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER <div style="text-align: center; font-weight: bold;">Z01 NS 01986-05 ID</div> |
| PERIOD COVERED <div style="text-align: center;">July 1, 1975 to June 30, 1976</div> | | |
| TITLE OF PROJECT (80 characters or less) Inoculation of Animals with Tissue Culture Grown Material from Patients with Chronic Neurological Diseases. | | |
| NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT | | |
| PI: | Dr. W.T. London Dr. A.E. Palmer Dr. D.A. Fuccillo Dr. J.L. Sever | Head, Exp. Path., IDB, NINCDS Res. Vet., Exp Path., IDB, NINCDS Head, Neuroviro., IDB, NINCDS Chief, IDB, NINCDS |
| Other: | Mrs. B.L. Curfman Mrs. G.M. Brown Mr. R.L. Brown | Biologist, Exp. Path., IDB, NINCDS Bio-Lab Tech (General) Exp. Path. IDB, NINCDS Bio-Lab Tech (animal) Exp. Path. IDB, NINCDS |
| COOPERATING UNITS (if any) Meloy Laboratories, Springfield, Virginia | | |
| LAB/BRANCH Infectious Diseases Branch | | |
| SECTION Section on Experimental Pathology | | |
| INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland, 20014 | | |
| TOTAL MANYEARS: | PROFESSIONAL: | OTHER: |
| 0.9 | 0.4 | 0.5 |
| SUMMARY OF WORK (200 words or less - underline keywords) The development of a non-human primate animal model for <u>subacute sclerosing panencephalitis</u> (SSPE) is in progress. It appears that <u>clinical signs of encephalitis</u> can be produced in immunosuppressed <u>cynomolgus monkeys</u> inoculated with a defective strain of SSPE virus. However, the course of the disease when clinical signs are first seen is so acute that the animals die before the typical symptoms of SSPE can develop. Animals are now on test that have not been immunosuppressed and should not have such an acute course of encephalitis. | | |

Project Description:

Objectives: The development of animal models to study the pathogenesis and treatment of certain chronic diseases of humans.

Methods Employed: The inoculation of various animal species with tissue culture grown material taken from human patients with chronic neurological diseases. The two diseases now under study are subacute sclerosing panencephalitis (SSPE) and multiple sclerosis (MS).

Immunosuppressive drugs will be used to increase the susceptibility of the animals to the agents. When animal models are developed, we can then test various new drugs (as they become available) as treatment for the diseases.

Major Findings: We have produced an acute form of measles encephalitis in cynomolgus monkeys by intracerebral inoculation of a defective SSPE virus (Niigata S/V strain). The virus was grown in Vero cell cultures and then inoculated into two monkeys previously immunosuppressed with cyclophosphamide. Seven to eight weeks later both animals developed signs of acute encephalitis and died eight days after onset of symptoms. When attempts were made to isolate the SSPE virus from these monkeys, it was discovered that the inoculum had been heavily contaminated with PPL0 bacteria. Work is now in progress to remove this organism from the virus-cell culture line by cloning.

Another SSPE strain "Biken" has been inoculated into measles susceptible African Green monkeys. This virus strain has been reported to produce SSPE-like disease in these monkeys. This virus has an incubation period of four weeks in the animals and then a much longer period of clinical illness. Inoculated animals are now under observation.

Significance of the Program to the Institute: A short time ago, the hypothesis that chronic or subacute degenerative diseases of the nervous system might be transmissible would have been an unbelievable conception to most medically trained pathologists or neurologists. To date, using animal models, at least two chronic degenerative conditions seen in humans have been transmitted. As these diseases become more fully understood, we may have new ideas for the study of other degenerative diseases of the brain and central nervous system. Using animal models infected with these agents a variety of drugs can be tested as treatment for these diseases.

Proposed Course of the Project: We will continue to try to develop a true animal model for SSPE, that is, an animal that has prolonged clinical signs of the disease. This model could then be used to test various drugs for treatment of SSPE.

Recently studies have been started with the multiple sclerosis associated agent (MSAA) in the PAM tissue culture cell line and mice. If reported

findings are successfully confirmed in our branch, the agent will be inoculated into a variety of animals in attempts to produce clinical disease.

Publications:

Zook, B.C., London, W.T., Sever, J.L., Sauer, R.M.: Experimental Lead Paint Poisoning in Nonhuman Primates, I. Clinical Signs and Course. Journal of Medical Primatology. (In press.)

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|---|----------------------|---|--|-----|-----------------|------------------------------|--|-----------------|------------------------------------|--------|----------------|--------------------|--|--------------------|---|--|----------------|----------------------------------|
| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | | U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 NS 01987-05 ID | | | | | | | | | | | | | | | |
| PERIOD COVERED July 1, 1975 to June 30, 1976 | | | | | | | | | | | | | | | | | | |
| TITLE OF PROJECT (80 characters or less) Caloric and Protein Restriction in Pregnancy and Its Effect on Newborn Rhesus Monkeys. | | | | | | | | | | | | | | | | | | |
| NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT <table border="0"> <tr> <td>PI:</td> <td>Dr. W.T. London</td> <td>Head, Exp. Path. IDB, NINCDS</td> </tr> <tr> <td></td> <td>Dr. A.E. Palmer</td> <td>Res. Vet., Exp. Path., IDB, NINCDS</td> </tr> <tr> <td>Other:</td> <td>Dr. J.L. Sever</td> <td>Chief, IDB, NINCDS</td> </tr> <tr> <td></td> <td>Dr. J.H. Ellenberg</td> <td>Acting Head, Math.Stat., OB & E, NINCDS</td> </tr> <tr> <td></td> <td>Dr. J.G. Bieri</td> <td>Chief, Nut.Biochem., LNE, NIAMDD</td> </tr> </table> | | | | PI: | Dr. W.T. London | Head, Exp. Path. IDB, NINCDS | | Dr. A.E. Palmer | Res. Vet., Exp. Path., IDB, NINCDS | Other: | Dr. J.L. Sever | Chief, IDB, NINCDS | | Dr. J.H. Ellenberg | Acting Head, Math.Stat., OB & E, NINCDS | | Dr. J.G. Bieri | Chief, Nut.Biochem., LNE, NIAMDD |
| PI: | Dr. W.T. London | Head, Exp. Path. IDB, NINCDS | | | | | | | | | | | | | | | | |
| | Dr. A.E. Palmer | Res. Vet., Exp. Path., IDB, NINCDS | | | | | | | | | | | | | | | | |
| Other: | Dr. J.L. Sever | Chief, IDB, NINCDS | | | | | | | | | | | | | | | | |
| | Dr. J.H. Ellenberg | Acting Head, Math.Stat., OB & E, NINCDS | | | | | | | | | | | | | | | | |
| | Dr. J.G. Bieri | Chief, Nut.Biochem., LNE, NIAMDD | | | | | | | | | | | | | | | | |
| COOPERATING UNITS (if any) Royal Childrens' Hospital, Victoria, Australia, 3052. | | | | | | | | | | | | | | | | | | |
| LAB/BRANCH Infectious Diseases Branch | | | | | | | | | | | | | | | | | | |
| SECTION Section on Experimental Pathology | | | | | | | | | | | | | | | | | | |
| INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland, 20014 | | | | | | | | | | | | | | | | | | |
| TOTAL MANYEARS: 0.4 | PROFESSIONAL: 0.2 | OTHER: 0.2 | | | | | | | | | | | | | | | | |
| SUMMARY OF WORK (200 words or less - underline keywords) A group of 24 pregnant rhesus monkeys have been maintained on one of three purified diets throughout pregnancy. One diet was restricted in protein, one deficient in both protein and calories and one was the control diet. The monkeys were delivered at 158 days of gestation by cesarean section. The fetuses were killed at time of delivery and all organs weighed and anthropometric measurements taken. Analysis of anthropometric data did not show significant differences between the fetuses taken from mothers on various diets. Furthermore analysis of chemical determinations of the cerebrum and cerebellum from fetuses of monkeys on the diets revealed no statistically significant changes in these tissues. These results indicate that the fetus of the Rhesus monkey is protected during frank <u>protein-calorie restriction</u> of the mother. This project has been terminated. | | | | | | | | | | | | | | | | | | |

Project Description:

Objectives: Initiate a nutritional study of non-human primates using pregnant rhesus monkeys to test the hypothesis that there is no causal relation between maternal nutrition during pregnancy and certain sensory, pathological, immunological and biochemical characteristics of the infant.

Methods Employed: In the nonhuman primate nutritional study, pregnant rhesus monkeys were maintained throughout pregnancy on one of three purified diets. One diet was restricted in protein, one deficient in both protein and calories, and the third was the control diet. The pregnant animals were delivered at 158 days gestation by cesarean section and the infant's tissues were processed for biochemical analysis. The nutritionally deprived female monkeys were continued on their respective diets and studies for immunological responses to the following seven antigens: Rubeola (wild strain), Mumps-Rubella vaccine, Diphtheria-Tetanus toxoid, and Influenza A as well as Venezuelan Equine Encephalomyelitis vaccines were conducted.

Major Findings: Twenty-four viable fetuses were delivered from monkeys maintained on one of three diets:

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| Group 1 - Control diet | (8 fetuses) |
| Group 2 - Protein deficient-calorie sufficient diet | (8 fetuses) |
| Group 3 - Calorie deficient-protein deficient diet | (8 fetuses) |

All organs were weighed at sacrifice and anthropometric measurements taken. When the fetal measurements (body weight, crown-rump, crown-heel and head circumference) were compared, no significant differences were found among the fetuses from monkeys maintained on the three diets.

The cerebrum and cerebellum were analyzed chemically to assess composition and growth. Analyses revealed no statistically significant changes in protein, DNA, RNA, cholesterol, phospholipid water or chloride space of either tissue.

These results indicate that the brain of the fetus of the Rhesus monkey is protected during frank protein-calorie restriction of the mother. Furthermore, it is during this time that the major part of brain development takes place.

No significant changes in the immunological response were noted except with Tetanus toxoid. The antibody titers for Tetanus were much lower in animals on the restricted protein-calorie diet than were the controls or the animals receiving restricted protein-normal calories.

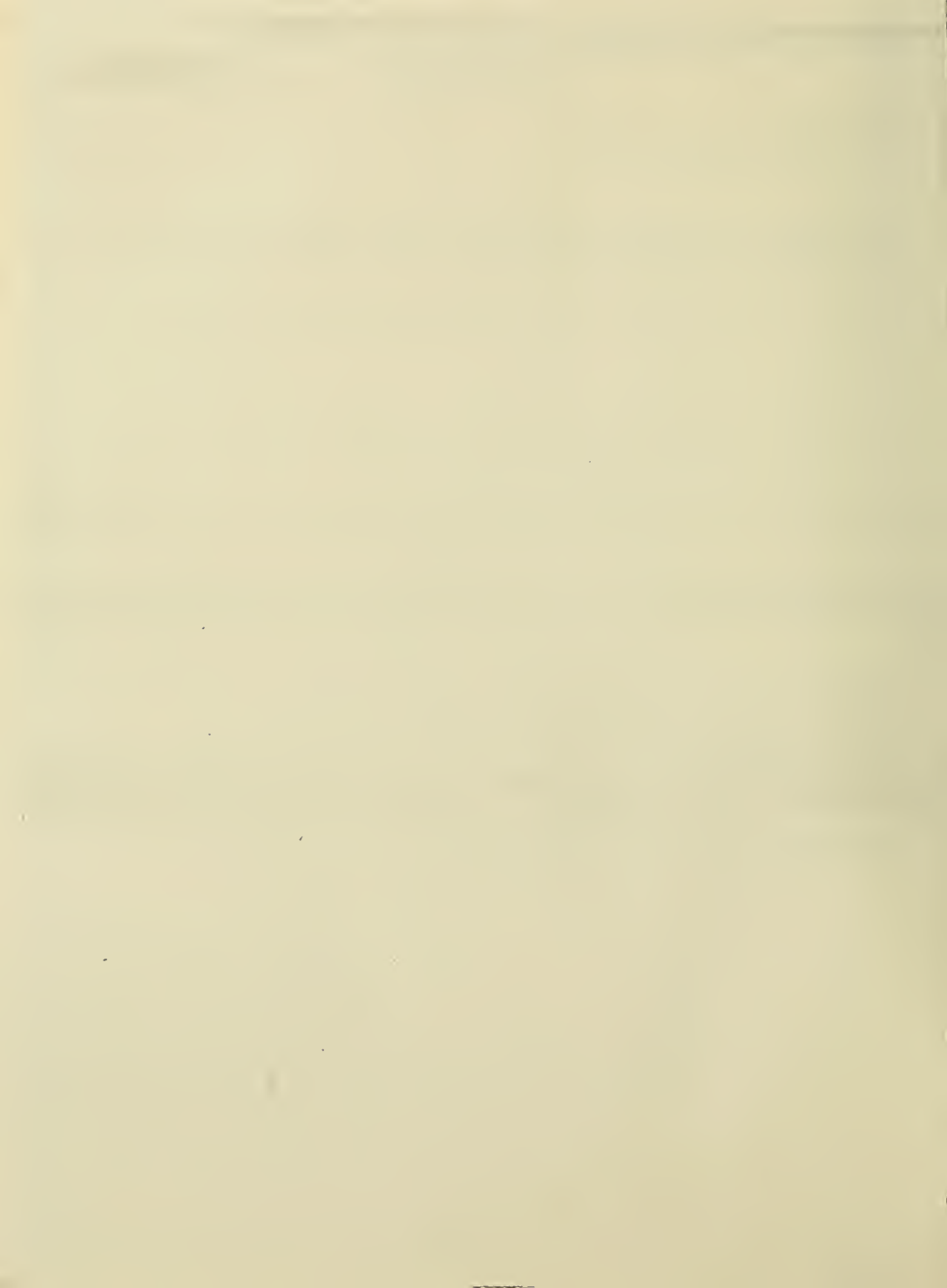
Significance of the Program to the Institute: General obstetric practice in the United States is to restrict weight gain during pregnancy except where specifically contraindicated. Investigators analyzing the maternal weight gain/birthweight data from the Perinatal Research Program have concluded that this practice may be actively increasing the low birthweight rate, and possibly the perinatal mortality rate. Indeed, this practice may produce babies who are smaller than expected for gestation and who may be poor performers post-natally and of lower intelligence.

Proposed Course of the Project: This project has been terminated.

Publications:

Cheek, D.B., Holt, A.B., London, W.T., Ellenberg, J.H., Hill, D.E., and Sever, J.L.: Nutritional Studies in the Pregnant Rhesus Monkey - The Effect of Protein-Calorie or Protein Deprivation on Growth of the Fetal Brain. American Journal of Clinical Nutrition (In Press).

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| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 NS 01988-05 ID |
| PERIOD COVERED July 1, 1975 to June 30, 1976 | | |
| TITLE OF PROJECT (80 characters or less) Herpes Virus Induction of Cervical Cancer in Cebus Monkeys. | | |
| NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT D.L. Sly Project Director, Meloy Laboratories, Springfield, Virginia | | |
| COOPERATING UNITS (if any) | | |
| LAB/BRANCH Infectious Disease Branch, Intramural Research | | |
| SECTION Section on Experimental Pathology | | |
| INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland, 20014 | | |
| TOTAL MANYEARS: | PROFESSIONAL: | OTHER: |
| SUMMARY OF WORK (200 words or less - underline keywords) This project is being done under Contract N01 NS 2 2306 | | |



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| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 NS 01989-05 ID |
| PERIOD COVERED July 1, 1975 to June 30, 1976 | | |
| TITLE OF PROJECT (80 characters or less) Transmission of Hepatitis B Virus to Subhuman Primates. | | |
| NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT PI: Dr. W.T. London Head, Exp. Path., IDB, NINCDS Dr. R.H. Purcell Head, Hepatitis Virus, LID, NIAID Other: Mrs. B.L. Curfman Biologist, Exp. Path., IDB, NINCDS | | |
| COOPERATING UNITS (if any) None | | |
| LAB/BRANCH Infectious Diseases Branch | | |
| SECTION Section on Experimental Pathology | | |
| INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland, 20014 | | |
| TOTAL MANYEARS: 0.4 | PROFESSIONAL: 0.4 | OTHER: 0.0 |
| SUMMARY OF WORK (200 words or less - underline keywords) <p>Type A Hepatitis has been extensively studied in susceptible chimpanzees. To date, the chimpanzee remains the species most susceptible to infection with this virus. Attempts to infect other <u>non-human primate</u> species with this virus are in progress.</p> <p>Studies of <u>type B Hepatitis</u> have also been carried out in chimpanzees. Vaccinated animals rechallenged with live Hepatitis B virus showed solid immunity to the infection. Saliva from three patients that were chronic carriers of B Hepatitis were pooled and inoculated into a chimpanzee. This animal developed type B Hepatitis, proving that non-percutaneous spread of Hepatitis B virus is possible.</p> <p>This project in collaboration with NIAID is now terminated.</p> | | |

Project Description:

Objectives: The study and characterization of Hepatitis A and B in non-human primates.

Methods Employed: Different species of nonhuman primates are inoculated (orally or parenterally) with material containing hepatitis A or B agents. The animals are monitored for serological, biochemical, and histopathological evidence of infection.

Major Findings: Type A hepatitis has been extensively studied in susceptible chimpanzees and attempts to infect other nonhuman primate species with this virus are in progress. To date, the chimpanzee remains the species most susceptible to infection with this virus.

Two chimpanzees previously experimentally infected with type A hepatitis were rechallenged; both animals demonstrated an anamnestic immune response to hepatitis A antigen, but neither developed evidence of illness. The hyperimmune serum resulting from this challenge experiment is currently being evaluated for use as an immunofluorescence and radioimmunoassay reagent.

Extensive studies of type B hepatitis have also been carried out. Chimpanzees vaccinated 18 months previously were rechallenged with live hepatitis B virus; solid immunity to infection was demonstrated.

To determine if body fluids other than blood from patients with type B hepatitis are infectious, saliva from three such patients was pooled and inoculated into a chimpanzee. This animal developed type B hepatitis, proving that non-percutaneous spread of hepatitis B virus via saliva may be epidemiologically important in the transmission of this disease. Similar studies are being carried out with semen from hepatitis B patients in an attempt to confirm the epidemiologically suspected venereal spread of hepatitis B virus. Studies have also begun on methods of terminating chronic hepatitis B virus infection, a problem that afflicts one out of a thousand individuals in the United States and as high as one out of ten persons in underdeveloped countries. Studies are being carried out in chronically infected chimpanzees. One such study, utilizing a stable form of the interferon inducer, polyriboinosinic polyribocytidylic acid (poly I:C) complexed with poly L lysine is near completion. Although hepatitis B virus infection was not terminated, marked changes in several parameters of infection were observed, indicating that synthesis of hepatitis B virus was suppressed during treatments. Studies of other antiviral agents are planned. The development of sensitive serologic tests for hepatitis A and B viruses has resulted in the identification of a third type of human viral hepatitis, not caused by either of these two viruses. This disease, provisionally designated "non-A, non-B" hepatitis accounts for as much as 25% of clinical viral hepatitis seen in the United States. Attempts to transmit this newly recognized disease entity to nonhuman primates are in progress, but to date, these studies have been unsuccessful.

Significance of the Program to the Institute:

This study developed in part as the result of serological surveys in the perinatal research program. It is no longer in the realm of the Institute.

Proposed Course of the Project:

This project in collaboration with NIAID is now terminated.

Publications:

Barker, L.F., Maynard, J.E., Purcell, R.H., Hoofnagle, J.H., Berquist, K.R., London, W.T.: Viral Hepatitis, Type B, in Experimental Animals. The American Journal of the Medical Sciences. 270:189-195, 1975.

Barker, L.F., Maynard, J.E., Purcell, R.H., Hoofnagle, J.H., Berquist, K.R., London, W.T., Gerety, R.J., Krushak, D.H.: Hepatitis B Virus Infection in Chimpanzees: Titration of Subtypes. The Journal of Infectious Diseases. 132: 451-458, 1975.

Dienstag, J.L., Feinstone, S.M., Purcell, R.H., Hoofnagle, J.H., Barker, L.F., London, W.T., Popper, H., Peterson, J.M., Kapikian, A.Z.: Experimental Infection of Chimpanzees with Hepatitis A Virus. The Journal of Infectious Diseases. 132: 532-545, 1975.

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| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 NS 02037-04 ID |
| PERIOD COVERED July 1, 1975 to June 30, 1976 | | |
| TITLE OF PROJECT (80 characters or less) Perinatal Carcinogenesis In <u>Erythrocebus Patas</u> Monkeys | | |
| NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT | | |
| PI: | Dr. W.T. London Dr. A.E. Palmer Dr. J.M. Rice | Head, Exp. Path., IDB, NINCDS Res. Vet., Exp. Path., IDB, NINCDS Senior Scientist, Exp. NCI |
| Others: | Mrs. B.L. Curfman | Biologist, Exp. Path. IDB, NINCDS |
| COOPERATING UNITS (if any) Meloy Laboratories, Springfield, Virginia | | |
| LAB/BRANCH Infectious Disease Branch | | |
| SECTION Experimental Pathology Section | | |
| INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland, 20014 | | |
| TOTAL MANYEARS: | PROFESSIONAL: | OTHER: |
| SUMMARY OF WORK (200 words or less - underline keywords) Transplacental carcinogenesis studies in the Patas monkey have shown that pregnant animals and their fetuses tolerate up to 12 sequential intravenous injections of <u>ethyl nitrosourea</u> (ENU) at a single-dose level of 0.1 mmole ENU/kg maternal body weight. However, no tumors of any kind have yet been observed in monkeys exposed to ENU in utero and observed up to 24 months after birth. Transplacental passage of ENU-14C is unimpeded by the primate placenta: blood levels in both mother and fetus reach identical maxima and follow the same kinetics after IV injection of the pregnant female. The primate placenta is thus no barrier to the potent transplacental carcinogen, ENU, and the location and severity of acute lesions resulting from intra-uterine exposure to this compound support the prediction that the primate nervous system will prove vulnerable to this agent. | | |
| This project has been incorporated into project No. Z01 NS 00972-05 ID. | | |

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| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 NS 02068-03 ID |
| PERIOD COVERED July 1, 1975 to June 30, 1976 | | |
| TITLE OF PROJECT (80 characters or less) Simian Hemorrhagic Fever in Patas and Rhesus Monkeys | | |
| NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT | | |
| PI: | Dr. W.T. London Dr. A.E. Palmer Dr. J.L. Sever | Head, Exp. Path, IDB, NINCDS Res. Vet., Exp. Path., IDB, NINCDS Chief, IDB, NINCDS |
| Other: | Dr. D.A. Fuccillo Dr. D.L. Madden Dr. H.B. Levy Mrs. B.L. Curfman Mr. R.L. Brown | Head, Neuroviral, IDB, NINCDS Head, Microbiol., IDB, NINCDS Head, Mol. Biol., LUD, NIAID Biologist, Exp. Path., IDB, NINCDS Bio Lab Tech (animal), Exp. Path., IDB, NINCDS |
| COOPERATING UNITS (if any) None | | |
| LAB/BRANCH Infectious Diseases Branch | | |
| SECTION Section on Experimental Pathology | | |
| INSTITUTE AND LOCATION NINCDS | | |
| TOTAL MANYEARS: 1.0 | PROFESSIONAL: .5 | OTHER: .5 |
| SUMMARY OF WORK (200 words or less - underline keywords) Simian hemorrhagic fever (SHF) is an enzootic disease in feral <u>E. patas monkeys</u> (10%). The agent has been found occasionally in other feral African <u>monkeys</u> . The disease among <u>Asian Macaque monkeys</u> is caused by a laboratory accident, usually transmitted by contaminated needles. SHF in patas monkeys can be mechanically transmitted by insect vectors. The virus from patas monkeys has not been isolated in tissue culture. The chronic disease in patas monkeys causes immune-complexes in the animal's kidneys and is vertically transmitted. Treatment was not successful when poly IC-poly-L-lysine complex was given to chronically infected patas monkeys. | | |
| This project has been terminated. | | |

Project Description:

Objectives: Simian hemorrhagic fever (SHF) is an acute, highly fatal disease which occurs in severe epizootics among Asian macaque monkeys. The virus responsible has been found in blood serum from clinically normal African monkeys. Under certain conditions one strain of the virus causes chronic disease among patas monkeys (*Erythrocebus patas*). This strain of the virus has not been grown in cell or tissue culture, thus its presence can only be determined by the inoculation of macaque monkeys who develop a rapidly progressing disease that is indistinguishable from that caused by other strains of SHF virus.

A reliable serological test to detect both antibodies and antigen is needed to identify infection in the feral patas monkey. There are at least two strains of SHF virus. One strain (LVR) has been isolated in cell culture from epizootics in Rhesus monkeys at NIH and at Sukhumi, USSR, in 1964.

Strain involved in the 1972 rhesus monkey and 1973 patas monkey outbreaks at NIH have not been isolated in cell culture. This must be done before the agents can be conclusively identified. This disease presents an infectious disease model in which resembles 1) immune complex diseases (such as lymphocytic choriomeningitis (LCM), 2) disseminated intravascular coagulopathy (DIC), and 3) the hemorrhagic fevers of man. We intend to study SHF in patas and rhesus monkeys in an attempt to better characterize its pathogenesis, prevention and treatment.

Methods Employed: There is presently no procedure available to detect the carrier state in clinically normal patas monkeys other than the inoculation of macaque monkeys. We are making progress toward the development of laboratory diagnostic techniques for this purpose. We have attempted immuno-diffusion, complement fixation and fluorescent antibody.

We are also exploring the following aspects of the disease:

1. Continue attempts to cultivate the causative agent on cell or tissue culture, and other animals such as nude mice.
2. Determine whether there is a virus-antibody complex in the serum of infected patas monkeys and, if so, whether this complex is responsible for the disease.
3. Determine if the disease is vertically transmitted in patas monkeys by following the offspring of chronically infected monkeys to determine if they are infected at birth or if they might become infected while nursing their mothers.
4. Susceptibility of patas monkeys to SHF.
5. Mode of transmission of SHF in patas monkeys.

6. Treatment of chronic infection of SHF in patas.

Major Findings: Simian hemorrhagic fever is an endemic disease in feral E. patas monkeys (10%). The agent has been found occasionally in feral Papio cynocephalus and Cercopithecus aethiops monkeys.

The disease among Asian Macaque monkeys is caused by a laboratory accident, usually transmitted by contaminated needles. Once the disease is established in a Macaque colony, it is readily transmitted to other monkeys by direct contact, fomites, aerosol, or parenteral inoculations.

In the natural host, E. patas and other African species, the virus is not transmitted by direct contact. Parenteral administration must be used to transmit the disease among these species. In the natural state this virus is probably transmitted mechanically by insect vectors. We were able to do this in the laboratory using stable flies.

All attempts to isolate the SHF virus from Rhesus monkeys infected with virus from patas monkeys or directly from chronically infected patas monkeys has failed.

Deposits seen by histological sections indicates that there is immune complex in the glomeruli of the chronically infected patas monkey.

The disease in patas monkeys is vertically transmitted. Babies born to chronically infected mothers are resistant to challenge with SHF virus. However, offspring from mothers not chronically infected are susceptible to the virus challenge. Chronically infected babies have not been observed at birth. However, the sample size (3) is not large enough to rule out this possibility.

Treatment of animals chronically infected with polyriboinosinic-polyribocytidylic acid complexed with poly L lysine has not been successful.

Significance of the Program to the Institute: SHF offers a potential animal model which may permit research on several diseases of significance to humans.

Proposed Course of the Project: This project will be terminated.

Publications:

Levy, H.B., Baer, G., Baron, S., Buckler, C.E., Gibbs, C.J., Iadarola, M.J., London, W.T. and Rice, J.: A Modified Polyriboinosinic-Polyribocytidylic Acid Complex That Induces Interferon in Primates. The Journal of Infectious Diseases. 132:434-439, 1975.

London, W.T.: Epizootiology and Transmission of Total Simian Hemorrhagic Fever in Rhesus Monkeys. Nature (In Press)

Publications:

Levy, H.B., London, W.T., Fuccillo, D.A., Baron, S., and Rice, J.:
Prophylactic Control of Simian Hemorrhagic Fever Virus in Monkeys by an
Interferon Inducer, Poly I.Poly C-Poly-L-Lysine. Journal Infectious Diseases
(In Press).

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| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | | U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 NS 02136-02 ID |
| PERIOD COVERED July 1, 1975 to June 30, 1976 | | | |
| TITLE OF PROJECT (80 characters or less) Control of Infectious Diseases in Experimental Animals Using Biologicals and Chemotherapeutic Agents. | | | |
| NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT | | | |
| PI: | Dr. W.T. London Dr. A.E. Palmer | Head, Exp. Path., IDB, NINCDS Res. Vet. Exp. Path., IDB, NINCDS | |
| Other: | Dr. D.A. Fuccillo Dr. J.L. Sever Dr. J.W. Larsen Mrs. B.L. Curfman Mr. R.L. Brown Mrs. G.M. Brown | Head, Neurovirol., IDB, NINCDS Chief, IDB, NINCDS Guest Worker, IDB, NINCDS Biologist, Exp. Path, IDB, NINCDS Bio.Lab.Tech(Animal), Exp. Path., IDB, NINCDS Bio.Lab.Tech.(General) Exp.Path., IDB, NINCDS | |
| COOPERATING UNITS (if any) Meloy Laboratories Inc. Springfield, Virginia | | | |
| LAB/BRANCH Infectious Diseases Branch | | | |
| SECTION Section on Experimental Pathology | | | |
| INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland, 20014 | | | |
| TOTAL MANYEARS: 1.8 | PROFESSIONAL: 1.0 | OTHER: 0.8 | |
| SUMMARY OF WORK (200 words or less - underline keywords) <u>Biological</u> and <u>chemotherapeutic compounds</u> are investigated for their ability to <u>control infectious diseases</u> in <u>experimental animals</u> . Biologicals include hyperimmune sera, globulins of human origin, bacterins, vaccines, antibiotics and antiviral agents. Products are tested in experimentally infected animals. | | | |

Project Description:

Objectives: To study prophylactic and therapeutic agents for the prevention and control of infectious diseases. The testing of candidate vaccines as to their immunogenicity, communicability and safety in experimental animals.

Methods Employed: New chemotherapeutic agents which show promise are studied in appropriate experimental animals. The animals are inoculated with a known infectious agent, then a therapeutic regimen is started, using the test drug. Additional animals are prophylactically treated with the drug, then challenged with the infectious agent.

Biological agents are tested for their ability to protect animals against naturally occurring and experimentally produced infectious diseases. Newly developed vaccines will be tested in susceptible experimental animals. Vaccinated animals will be exposed to susceptible sentinel animals to determine communicability. Vaccinated animals will be challenged at appropriate times to determine the immunogenicity of the vaccine.

Major Findings: 1. Ointments containing either two or five percent phosphonoacetic acid were not superior to the ointment base without the drug in clearing experimentally produced genital lesions of Herpes Simplex Virus, type 2 in female cebus monkeys (Cebus spp).

2. Immune serum globulin of human origin was found to substantially reduce morbidity and mortality in newly imported rhesus monkeys (Macaca mulatta).

3. Lancefields Group B Streptococcus produces a peracute fatal meningo-encephalitis when inoculated intracerebrally into neonate rhesus monkeys. This disease is eliminated by treatment with penicillin three to five hours post inoculation. Studies of this disease using bacteria, bacterial products and antisera as prophylactic and therapeutic agents are underway.

4. An agent that predictably produces subacute sclerosing panencephalitis in macaque monkeys is being maintained for the study of chemotherapeutic agents for the treatment of this disease.

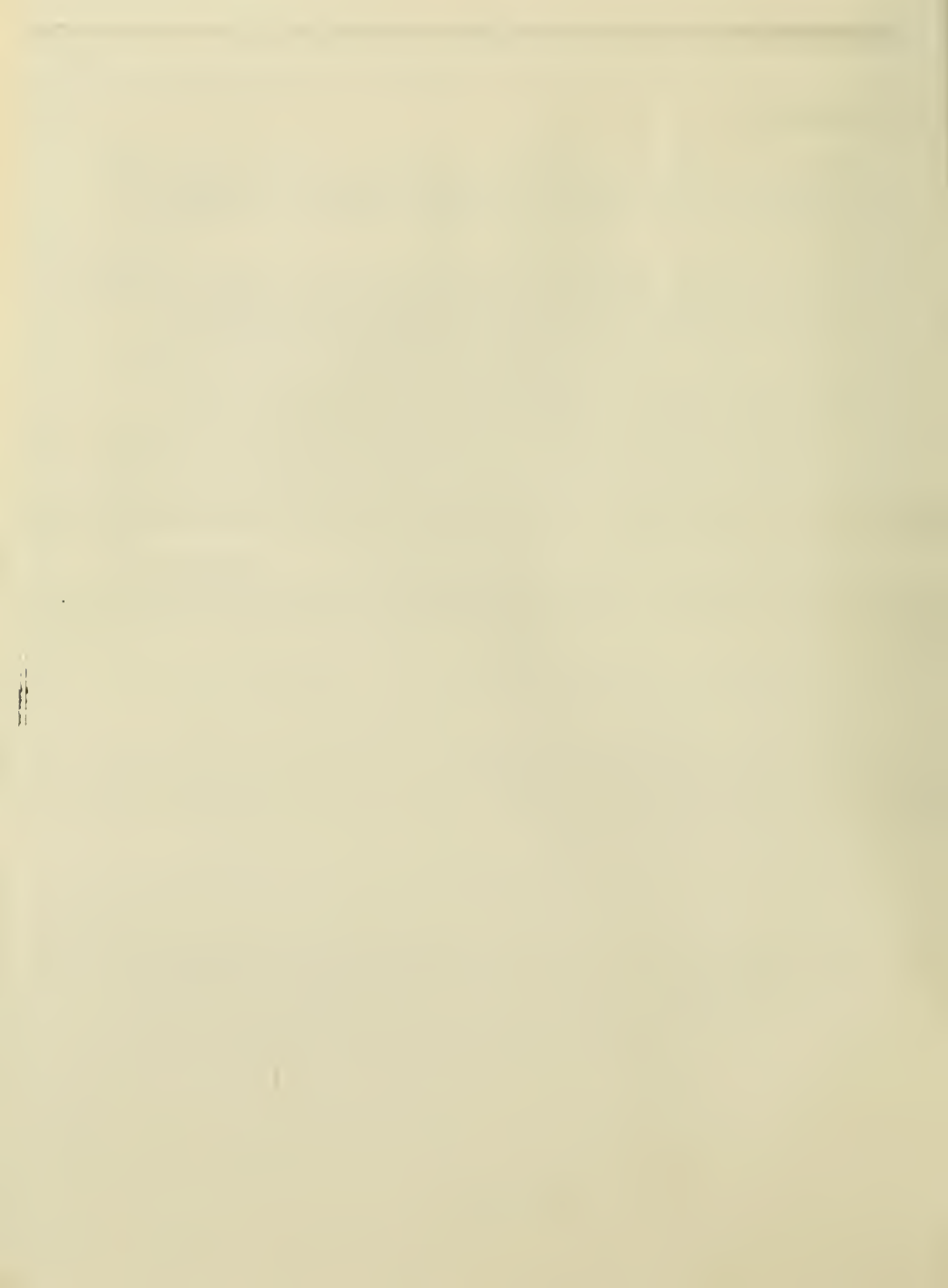
Significance of the Program to the Institute: Experimental animal studies permit the study of human diseases, their prevention and treatment with chemotherapeutic agents and biological products. Such studies provide information of efficacy, safety and side effects of these products. Information gained from experimental animal studies provides the bridge to the implementation of clinical studies in man.

Publications:

Palmer, A.E., London, W.T., Nahmias, A.J., Naib, Z.M., Tunca, J., Fuccillo, D.A., Ellenberg, J.H., Sever, J.L.: A Preliminary Report on Investigation of Oncogenic Potential of Herpes Simplex Virus Type 2 in Cebus Monkeys. Cancer Research 36: 807-809, 1976.

Wyatt, R.G., Sly, D.L., London, W.T., Palmer, A.E., Kalica, A.R., Van Kirk, D.H., Chanock, R.M., and Kapikian, A.Z.: Induction of Diarrhea in Colostrum-Deprived Newborn Rhesus Monkeys with the Human Reovirus-like Agent of Infantile Gastroenteritis. Archives of Virology 50: 17-27, 1976.

Barsky, D., Palmer, A.E., London, W.T., and Kerber, W.T.: Use of Immune Serum Globulin (Human) to Reduce Mortality in Newly Imported Rhesus Monkeys (Macaca mulatta). Medical Primatology. (In press.)



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| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 NS 02034-04 ID |
| PERIOD COVERED July 1, 1975 to June 30, 1976 | | |
| TITLE OF PROJECT (80 characters or less) Electron Microscopic Membrane Studies of Measles, SSPE and Other Paramyxoviruses. | | |
| NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT | | |
| PI: | Dr. Monique Dubois- Dalcq | Head, Sec. on Electron Microscopy |
| IDB, IRP, NINCDS | | |
| Other: | Dr. T. S. Reese | Head, Sec. on Functional Neuroanatomy |
| Dr. Michael Murphy Dr. David A. Fuccillo Ms. Kathy Worthington Mr. Otto Gutenson | Head, Unit on Biochemistry Head, Sec. on Neurovirology Histopathology Technician Biologist | LNNS, IRP, NINCDS IDB, IRP, NINCDS IDB, IRP, NINCDS IDB, IRP, NINCDS IDB, IRP, NINCDS |
| COOPERATING UNITS (if any) | | |
| None | | |
| LAB/BRANCH Infectious Diseases Branch | | |
| SECTION Electron Microscopy | | |
| INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Md. 20014 | | |
| TOTAL Person Years 1.3 | PROFESSIONAL: 0.9 | OTHER: 0.4 |
| SUMMARY OF WORK (200 words or less - underline keywords) The mechanism of <u>SSPE virus latency in vitro</u> has been analyzed using <u>freeze-fracture</u> , <u>surface replication</u> and <u>EM immunolabeling techniques</u> . In one carrier cell line, a peculiar defective <u>bud formation</u> has been detected and analyzed whereas in others a dissociation between cell fusion and virus production has been observed. The latter situation appears to be the closest to what happens in the brain. | | |

Project Description:

Objectives: To explore different latently infected cell lines and elucidate the mechanisms of viral latency with SSPE and other closely related viruses.

Methods Employed: 1) Pellets of fixed cells were frozen in glycerol and freeze-fractured in a Balzer freeze-etcher apparatus. Replicas of the fractured membrane were made and studied under EM, 2) monolayers of cells on coverslips were fixed and dehydrated. They were then either air dried or critical point dried and a replica of their surface was made in the Balzer freeze-etcher. In some cases the surface viral antigens were labeled by the EM immunoperoxidase technique as described previously (J. Virol. 12: 909-918, 1973).

Major Findings: A. Defective bud formation: Human prostate cells chronically infected with the Mantooth Strain of SSPE virus contained intracytoplasmic nucleocapsids but released only small amounts of infectious virus and were able to support replication of vesicular stomatitis virus. Most of these carrier cells lacked the round buds observed in productive cells but had instead many elongated processes attached to the cell surface. Each of these processes contained one or two hairpin ridges, each overlying a nucleocapsid. In this carrier cell line several defects appear to exist which depend on the specific interaction of a certain viral strain with a certain cell type. These defects prevent the deployment of viral antigen over the entire cell surface, the formation of nucleocapsids of normal length, the coiling of attached nucleocapsids and the consolidation of sheets of viral membrane into the spherical buds with the nucleocapsids coiled inside. These defects may account for the failure of carrier cells to shed infectious virus. B. Dissociation between propagation of infection by cell fusion and production of viral particles. In this case 2 different situations can be observed. The giant cells are covered with patches of diffuse antigen only and there is no stage preparatory for budding. This was observed in vero cells inoculated with a cell associated neutropic SSPE strain which was adapted to the monkey brain and provided to us by Dr. Albrecht, (FDA). In contrast, in cells derived from hamster brain chronically infected with SSPE Mantooth - HBS virus (provided by Dr. K. Johnson - UCSF), the giant cells are covered with viral antigen organized in strands as well as diffuse antigen on their surface and a small number of complete virus is produced at the edge of the cells. Finally, CV₁ cells latently infected with the 107 virus were provided to us by Dr. Bachmann (Munich, Germany). This virus has been isolated from sporadic Bovine Meningo encephalitis and appears to share some common antigen with SSPE. However, the structure and surface features of the giant cells produced by the 107 virus differed in several ways from SSPE infection.

Significance of the Program to the Institute : Immuno-freeze etching and surface replication combined with immunolabeling are new methods that allow studies of extensive areas of the membrane of infected cells as compared to controls. Using these methods, some mechanisms of the complete maturation of SSPE virus have been elucidated in productive cells, and found to be

dramatically altered in cells latently infected with similar viruses. Similar events might take place in the SSPE brain but analysis of their structural and molecular basis can be more easily done in vitro.

Proposed Course of the Project: The early stages of virus cell interaction will be studied with similar techniques. Lymphocyte receptors for paramyxovirus will be investigated in collaboration with Dr. H. McFarland.

Publications:

Dubois-Dalcq, M., and Reese, T. S.: Structural changes in the membrane of vero cells infected with a paramyxovirus. J. Cell Biol., 67: 551-565, 1975.

Dubois-Dalcq, M., Reese, T. S., Murphy, M. and Fuccillo, D.: Defective bud formation in human cells chronically infected with SSPE virus. J. Virol. In Press, 1976.

Dubois-Dalcq, M. In Meulen, V. Ter and Katz, M. (Eds.) Workshop on the Application of Modern Biological Research to Investigation of Slow Virus Infection of the CNS. Wurzburg, Germany. Springer, Verlag. In Press, 1976.

Dubois-Dalcq, M., Worthington, K., Gutenson, O. and Barbosa, L.H.: Immunoperoxidase labeling of subacute sclerosing panencephalitis (SSPE) virus in hamster acute encephalitis. Lab. Invest., 32: 518-526, 1975.

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| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 NS 02035-04 ID |
| PERIOD COVERED July 1, 1975 to June 30, 1976 | | |
| TITLE OF PROJECT (80 characters or less) Electron Microscopic Studies in Multiple Sclerosis Brain | | |
| NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT | | |
| PI: | Dr. Monique Dubois- Dalcq | Head, Sec. on Electron Microscopy |
| Other: | Ms. Kathy Worthington | Histopathology Technician |
| | | IDB, IRP, NINCDS IDB, IRP, NINCDS |
| COOPERATING UNITS (if any) Dr. C.S. Raine, Albert Einstein College of Medicine, Bronx, New York Dr. J. Prineas, New Jersey Medical School, New Jersey. | | |
| LAB/BRANCH Infectious Diseases Branch | | |
| SECTION Electron Microscopy | | |
| INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Md. 20014 | | |
| TOTAL Person Years 0.4 | PROFESSIONAL: 0.2 | OTHER: 0.2 |
| SUMMARY OF WORK (200 words or less - underline keywords) No <u>measles antigen</u> has been detected in Multiple Sclerosis from 6 well documented cases. <u>Freeze-fracture</u> studies of adequately fixed MS brain are in progress. | | |

Project Description:

Objectives: 1. To search measles antigen in the MS lesions.

2. To study the inner structure of membranes inside the plaques and periplaques.

Methods Employed: MS cases with active lesions were studied. For immunolabeling studies, formaldehyde fixed or frozen brain was used. Sections were incubated in SSPE globulin coupled to peroxidase. For freeze-fracturing glutaraldehyde fixed brain was impregnated with glycerol and frozen in freon. Plaque and periplaques areas were fractured and a replica made in the Balzer freeze etcher.

Major Findings: A. No measles antigen was detected in active brain lesions of 3 patients (which bring to 6, the number of MS brains negative for measles studied during the last 2 years). The same SSPE conjugate stained intensely measles viral antigen inside the nerve cells of organotypic nervous tissue cultures and in human brain from a SSPE patient. B. The freeze-fracture techniques has never so far been applied to human brain and will be only of interest if optimal conditions of fixation with minimal postmortem autolysis are obtained. In the first case studied, which was fixed 3 hours after death, structures characteristic of the astrocytic membrane (assemblies) had disappeared. However, aggregates of particles were recognized in the membrane of denuded axons. Cells identified as histiocytes appear to contain smooth lamellar inclusions and myelin figures. Further studies are in progress on another case where the brain was perfused with aldehydes 10 min. after death.

Significance of the Program to the Institute: Multiple Sclerosis is one of the most widespread neurological diseases among young people in this country and its etiology is still unknown. Recent reports have suggested that MS may be associated with a viral infection (measles, in some cases). This may induce an autoimmune demyelination process.

Proposed Course of the Project: The freeze-fracture study will be pursued. If new brains are available, well controlled study of the IgG staining in the plaques should be done. Using guinea pig antisera against the MSAA (Henle, et al, Infection and Immunity, 1975) attempt to detect this agent in the brain will be performed using the indirect IP test. Membrane and immunolabeling studies on MS lymphocytes are projected in collaboration with the Neuroimmunology Branch.

Publications:

Dubois-Dalcq, M., Schumacher, G. and Worthington, K.: Immunoperoxidase studies on multiple sclerosis brain. Abstract, "Perspectives in Multiple Sclerosis", Neurology, 25: 496, 1975.

Raine, C.S., Prineas, J.W., Sheppard, R.D., Bernstein, M.B. and Dubois-Dalcq, M.: Immunohistochemical tests for measles antigens in multiple sclerosis and measles infected CNS tissue. Jour. of Neuropath. and Exp. Neurol. (35)3: 300-384, May-June, 1976.

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| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 NS 02199-01 ID |
| PERIOD COVERED July 1, 1975 to June 30, 1976 | | |
| TITLE OF PROJECT (80 characters or less) Electron Microscopic Membrane Studies of Slow Viruses of the Nervous System. | | |
| NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT | | |
| PI: Other: | Dr. Monique Dubois- Dalcq Dr. T. S. Reese Dr. C. Gibbs Dr. D.C. Gajdusek Dr. D.A. Fuccillo Dr. M. Rodriguez Ms. K. Worthington | Head, Sec. on Electron IDB, IRP, NINCDS Microscopy Head, Sec. on Functional LNNS, IRP, NINCDS Neuroanatomy Asst. Chief, Lab. Central CNS, IRP, NINCDS Nervous System Studies Chief, Lab. Central CNS, IRP, NINCDS Nervous System Studies Head, Sec. on Neurovirology IDB, IRP, NINCDS Postdoctoral Fellow IDB, IRP, NINCDS Histopathology Technician IDB, IRP, NINCDS |
| COOPERATING UNITS (if any) Dr. O. Narayan, Dept. of Neurology, Neurovirology Lab., Johns Hopkins University School of Medicine Dr. A. Haase, Dept. of Medicine, School of Medicine, University of California | | |
| LAB/BRANCH Infectious Diseases Branch | | |
| SECTION Electron Microscopy | | |
| INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Md. 20014 | | |
| TOTAL Person Years 2 | PROFESSIONAL: 1.6 | OTHER: 0.4 |
| SUMMARY OF WORK (200 words or less - underline keywords) Structural changes in the membrane of cells infected respectively with <u>scrapie</u> , <u>visna</u> and <u>herpes</u> viruses have been identified using <u>freeze-fracture</u> , <u>surface</u> <u>replication</u> and <u>immunolabeling techniques</u> . In the scrapie brain, abnormal features were observed in the neural and astrocytic membranes of clinically af- fected animals. In visna infected cells, the virus maturation was characterized by the insertion and growth of surface units probably representing viral anti- gens, by the reorganization of the host membrane proteins under these units and by the attachment of a coiled capsid to the modified membrane. In herpes infection, unusual groups of tightly packed particles were identified in the cleaved membrane at the sites where viral antigen is probably incorporated. | | |

Project Description:

Objectives: To study under EM structural changes in the membrane of cells infected respectively with scrapie, visna, and herpes viruses.

Methods Employed: A. Freeze-Fracture: For the scrapie study, clinically affected mice as well as subclinical animals and appropriate controls, were perfused with aldehyde fixatives through the heart. For the visna and herpes studies, infected cells were fixed in aldehydes and pelleted. Fixed brain slices and pellets were frozen in glycerol and freeze-fractured in a Balzer freeze-etcher apparatus. Replicas of the fractured membrane were then studied under EM.

B. Surface Replica: monolayers of visna infected cells were fixed, dehydrated and critical point dried. A replica of their surface was then made in the Balzer freeze etcher.

C. Immunolabeling: Visna infected monolayers were fixed and viral antigen was labeled with a serum against P30, the major core polypeptide, in the indirect immunoperoxidase test. Unfixed Herpes I infected cells were stained with herpes antiserum for the detection of surface antigen in the indirect immunofluorescence test.

Major Findings: A. Scrapie: In the freeze-fractured scrapie cerebellum, the innermost limiting membrane of vacuole walls lacked the intramembrane particles present on normal neuronal and glial membranes. This might represent a change in the organization or distribution of the membrane proteins. Astrocytes around the vacuoles were characterized by an increased number of "assemblies." These changes probably represent an aspect of the astrocytic hypertrophy known to occur in scrapie. Structural abnormalities were also observed in membranes at some distance of the vacuoles and the unusual fracturing pattern suggested that abnormal adherence might exist between cells, may be at the point where the infection is spreading from one cell to the next. However no new structure likely to be the scrapie agent itself has been uncovered by the freeze-fracture techniques.

B. Visna: Membrane changes associated with the different steps of visna assembly have been identified. Early membrane changes consist of flat regions which lack intramembrane particles and show clusters of globular units on corresponding areas of the surface. The capsid, which is labeled by the P30 serum, seems to initiate the budding process when it attaches to these modified membrane regions. The surface units can move laterally and behave as if they were anchored to the capsid, and formed membrane complexes. Surface units constitute a marker for viral structures and probably correspond to viral antigen present in clusters of spikes on the visna virus surface. Final maturation occurs after the virus is released from the cell and consists of capsid uncoiling and reduction in size of the viral envelope.

C. Herpes: Using the freeze-fracture techniques, particles in increased density (PID) have been identified on the inner leaflet of the viral envelope. The population of intramembrane particles is three times larger

on PID areas than on the control plasmalemma. PID areas were also seen on budding sites detected on the nuclear membrane, on proliferating membranes and on the plasma membranes of the infected cells. The PID areas detected by freeze-fracture might thus represent viral antigen and constitute a new marker for herpes infection.

Significance of the Program to the Institute: A. Scrapie is a model for the transmissible spongiform encephalopathies in human; the morphology of the virus and its intimate relationship with the cell membrane has not yet been structurally defined. B. Visna is a demyelinating disease of the sheep in which the virus in the brain is in the provirus state, a situation that might well happen in multiple sclerosis. C. It is not known whether neural membranes are modified during latency or reactivation with herpes viruses. All these problems are thus interesting to study using EM membrane techniques combined with the detection of viral antigen.

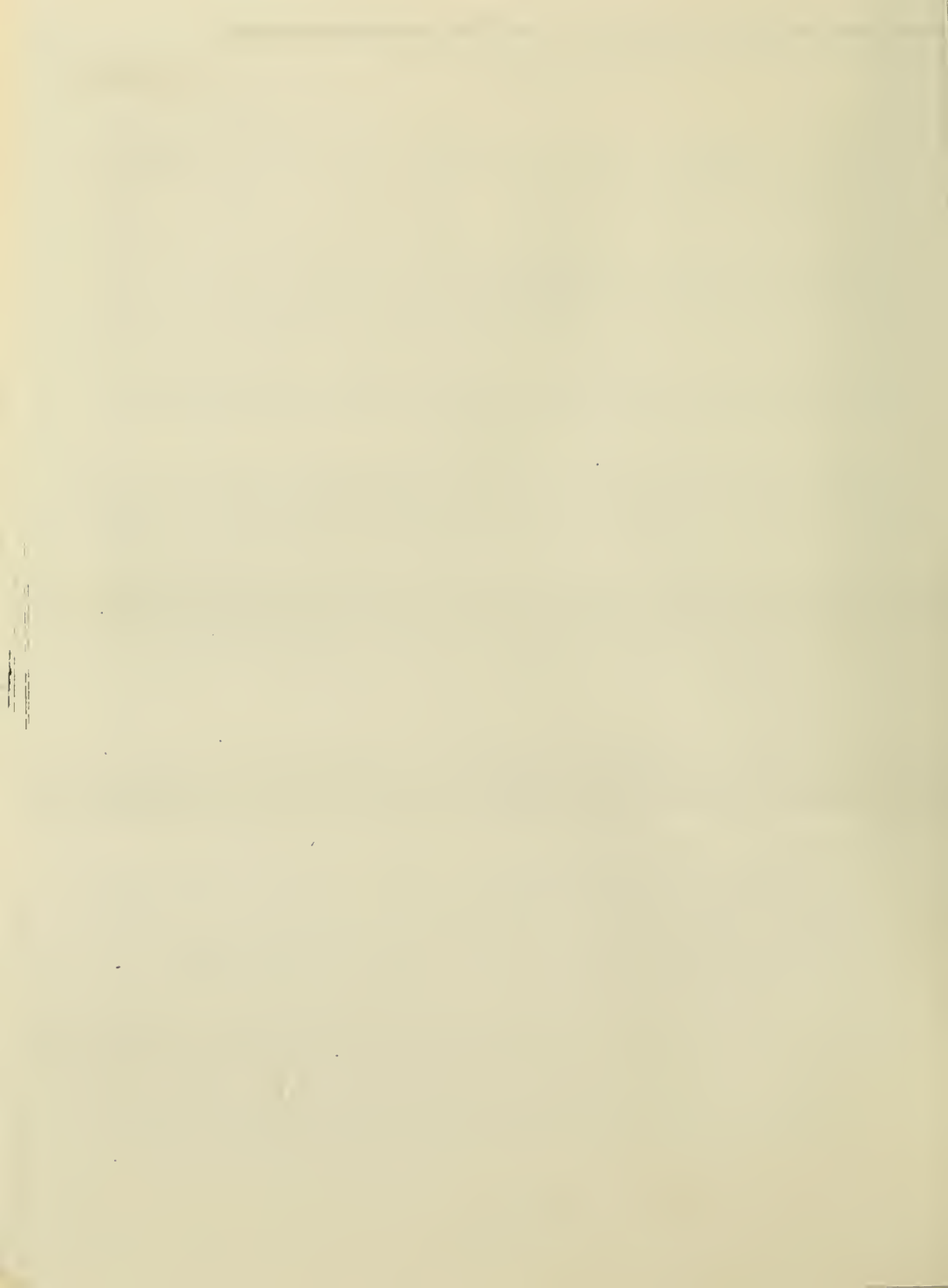
Proposed Course of the Project : A. A virus like particle, only 14 nm large, has been recently obtained in high number from geontron treated scrapie brain (Cho H.J. et al, Nature 257: 685, 1975). Fractions obtained by Dr. Gibbs and co-workers will be screened by negative staining to confirm or infirm this important observation. B. The localization of the P30 antigen and the surface antigen in visna infected cells will be correlated with the changes in shape and the number of budding sites observed by scanning EM. Also we will attempt to detect the P30 antigens in visna sheep brains. C. A sequential study will be performed to attempt to correlate the DIP areas in herpes infected cells with the presence of viral antigen on the nuclear and plasma membranes.

Publications :

Dubois-Dalcq, M. In Meulen, V. Ter and Katz, M. (Eds.) Workshop on the Application of Modern Biological Research to Investigation of Slow Virus Infection of the CNS. Wurzburg, Germany. Springer, Verlag. In Press, 1976.

Dubois-Dalcq, M., Reese, T.S., Haase, H. Rodriguez, M. and Narayan, O.: Membrane changes associated with assembly of visna virus. Jour. of Neuropath. and Exp. Neurol. (35)3: 300-384, May-June, 1976.

Rodriguez, M., Dubois-Dalcq, M. and Fuccillo, D.A.: Freeze-fracture study of herpes virus infection in vitro. Jour. of Neuropath. and Exp. Neurol. (35)3: 300-384, May-June, 1976.



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| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 NS 01924 06 ID |
| PERIOD COVERED <div style="text-align: center; margin-top: 5px;">July 1, 1975 to June 30, 1976</div> | | |
| TITLE OF PROJECT (80 characters or less) <div style="text-align: center; margin-top: 5px;">Genetic Studies of the Torsion Dystonias and Other Disorders of Movement</div> | | |
| NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT | | |
| PI: OTHER: | R. Eldridge M. Ziegler C.R. Lake A. Dekaban T. Koerber | Head, Section on Neurogenetics Research Associate Research Associate Associate Chief, Developmental and Metabolic Neurology Branch Research Assistant |
| | | IDB, NINCDS LCS, NIMH LCS, NIMH DMN, NINCDS IDB, NINCDS |
| COOPERATING UNITS (if any) | | |
| Laboratory of Clinical Science, NIMH, NIH Developmental and Metabolic Neurology Branch, NINCDS, NIH | | |
| LAB/BRANCH <div style="text-align: center; margin-top: 5px;">Infectious Disease Branch</div> | | |
| SECTION <div style="text-align: center; margin-top: 5px;">Section on Neurogenetics</div> | | |
| INSTITUTE AND LOCATION <div style="text-align: center; margin-top: 5px;">NINCDS, NIH, Bethesda, Maryland 20014</div> | | |
| TOTAL MANYEARS: <div style="text-align: center; margin-top: 5px;">1.125</div> | PROFESSIONAL: <div style="text-align: center; margin-top: 5px;">.375</div> | OTHER: <div style="text-align: center; margin-top: 5px;">.75</div> |
| SUMMARY OF WORK (200 words or less - underline keywords) | | |
| <p> In this project we seek to clarify and expand the <u>nosology</u> of the hereditary <u>movement disorders</u>, contribute to their understanding of their underlying biochemical basis, determine the most effective <u>treatment</u> for each, and suggest guidelines for <u>counseling</u> individuals at risk. General syndromes under study include the <u>dystonias</u>, <u>tic disorders</u> including <u>Tourette syndrome</u>, <u>Huntington chorea</u> and <u>myoclonus</u>. Approaches include standard <u>epidemiologic</u> and clinical <u>genetic</u> studies together with collaborative efforts in evaluating the role of <u>neurotransmitters</u> such as <u>norepinephrine</u>, <u>dopamine</u>, <u>GABA</u>, and related enzymes. </p> <p> Project Numbers 01925-05, 01977-04, 02113-02, 02114-02 and 02046-03 have been incorporated into Project Number Z01 01924-06 ID, shown above. </p> | | |

Project Description:

Objectives: Included among the disorders of movement such as the choreas, the dystonias, the myoclonic states and the tic syndromes are a number of discrete diseases which are due to single gene mutations. Examples of mutations producing autosomal dominant traits are Huntington's chorea and one form of torsion dystonia. Examples of mutations leading to autosomal recessive traits are Lafora type myoclonic epilepsy and the type of torsion dystonia found in the Jewish population.

It is the objective of this project to uncover additional specific diseases within general movement disorder syndromes, contribute to the understanding of their underlying biochemical basis, determine the most effective treatment for each, and suggest guidelines for counseling individual family members.

Methods Employed: The initial approach involves detailed, personal evaluation of individuals who exhibit a particular syndrome and their relatives as well. Extensive genealogic data is then analyzed in conjunction with the clinical observations and relevant laboratory and tissue studies. A nosologic classification is then prepared and promising biochemical leads are explored in collaboration with established investigators. Simultaneously, existing treatment programs are evaluated and, where indicated, therapeutic trials are done to evaluate new agents. The latter studies are "double blind" with drug levels monitored where possible.

Major Findings: Contributions were made in the following areas during FY 1976:

DYSTONIA: Demonstration of significant elevation in plasma norepinephrine in those with the autosomal dominant form of torsion dystonia¹ complementing our previously reported observation that dopamine beta hydroxylase is elevated in plasma; comprehensive classification of the dystonias including nine primary or hereditary forms and 15 secondary forms;² genetic and epidemiologic review based on 768 families;^{3,4} emphasis of the variable course of the hereditary and sporadic dystonias in contrast to the general impression that this group is uniformly fatal;⁵ co-editorship of a 38 chapter monograph devoted exclusively to the dystonias.⁶

HUNTINGTON CHOREA: Report on attitudes of patients and their relatives towards the symptoms of the disease and its genetic and social implications. Chorea was most troublesome to patients while mental changes were most troublesome to spouses. Many of those at risk would not take advantage of a predictive test if it were available.⁷

TOURETTE SYNDROME: Demonstration of a familial concentration together with apparently high Jewish incidence; history of frequent transient motor and vocal tics in female relatives of Tourette patients who tend to be male; an important behavioral component which very likely has a

biochemical basis; documentation of normal peripheral catecholamine metabolism. These are the subjects of three upcoming articles.

MYOCLONIC EPILEPSY: Based on our evaluation of over 40 individuals in 26 families there are at least five hereditary forms of progressive myoclonic epilepsy with dementia of which that described clinically and pathologically by LaFora is best known. This year six patients, all adolescents from two different families and all affected by a severe non LaFora type syndrome, were evaluated extensively in collaboration with Dr. Anatole Dekaban, Developmental and Metabolic Neurology Branch, NINCDS, NIH. Analysis of various tissues outside the central nervous system did not confirm previous suggestion of abnormal glycogen or mucopolysaccharide metabolism. Recently a seventh adolescent died after a seven year illness. Extensive metabolic studies of frozen tissues are underway at the Neurologic Institute, New York City and at NIH.

Significance to Biomedical Research and the Program of the Institute: Although most of these disorders affect no more than several thousand individuals, the exception being Huntington Disease in which 15,000 to 20,000 may be affected or at risk, in total they constitute a sizable public health problem. This is particularly true when relatives, who must live with or are at risk for the hereditary trait, are considered. In addition, information gained from analysis of these discrete genetic traits may contribute to the cause and treatment of related but more common problems such as Parkinsonism, idiopathic epilepsy and mental retardation.

Proposed Course: Continue search for distinct entities and their biochemical basis, specific therapy, and prevention.

- Publications:
- (1) Ziegler, M., Lake, C.R., Eldridge, R., and Kopin, I.: Plasma Norepinephrine and Dopamine β -Hydroxylase in Dystonia. In Eldridge, R., and Fahn, S. (Ed.): Advances in Neurology. New York, Raven Press, May 1976, pp. 307-318, vol. 14
 - (2) Fahn, S., and Eldridge, R.: Definition of Dystonia and Classification of the Dystonic States. In Eldridge, R., and Fahn, S. (Ed.): Advances in Neurology. New York, Raven Press, May 1976, pp. 1-5, vol. 14
 - (3) Eldridge, R., and Gottlieb, R.: The Primary Hereditary Dystonias: Genetic Classification of 768 Families and Revised Estimate of Gene Frequency, Autosomal Recessive Form, with Selected Bibliography. In Eldridge, R., and Fahn, S. (Ed.): Advances in Neurology. New York, Raven Press, May 1976, pp. 457-474, vol. 14
 - (4) Eldridge, R.: Edward Flatau, Wladyslaw Sterling, Torsion Spasm in Jewish Children, and the Early History of Human Genetics. In Eldridge, R., and Fahn, S. (Ed.):

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| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | | U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 NS 01925 06 ID |
| PERIOD COVERED July 1, 1975 to June 30, 1976 | | | |
| TITLE OF PROJECT (80 characters or less) Intelligence in Israeli Patients with Torsion Dystonia | | | |
| NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT PI: R. Eldridge Head, Section on Neurogenetics ID, NINCDS | | | |
| COOPERATING UNITS (if any) Department of Education, Herbert H. Lehman College, NY | | | |
| LAB/BRANCH Infectious Disease Branch | | | |
| SECTION Section on Neurogenetics | | | |
| INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20014 | | | |
| TOTAL MANYEARS: | PROFESSIONAL: | OTHER: | |
| SUMMARY OF WORK (200 words or less - underline keywords) This project has been incorporated into Project No. Z01 NS 01924 06 ID. | | | |

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| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 NS 01927 06 ID |
| PERIOD COVERED <p style="text-align: center;">July 1, 1975 to June 30, 1976</p> | | |
| TITLE OF PROJECT (80 characters or less) <p style="text-align: center;">Clinical, Genetic, Biochemical and Pathologic Study of Hereditary Nervous System Tumors</p> | | |
| NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT | | |
| PI: R. Eldridge OTHER: G. Guroff T. Koerber | Head, Section on Neurogenetics Chief, Section on Intermediary Metabolism Research Assistant | IDB, NINCDS LBS, NICHD IDB, NINCDS |
| COOPERATING UNITS (if any) <p style="text-align: center;">Laboratory of Biomedical Sciences, NICHD, NIH Department of Neurology, Albert Einstein College of Medicine, NY</p> | | |
| LAB/BRANCH <p style="text-align: center;">Infectious Disease Branch</p> | | |
| SECTION <p style="text-align: center;">Section on Neurogenetics</p> | | |
| INSTITUTE AND LOCATION <p style="text-align: center;">NINCDS, NIH, Bethesda, Maryland 20014</p> | | |
| TOTAL MANYEARS: <p style="text-align: center;">.375</p> | PROFESSIONAL: <p style="text-align: center;">.125</p> | OTHER: <p style="text-align: center;">.25</p> |
| SUMMARY OF WORK (200 words or less - underline keywords) | | |
| <p>In this project we seek: <u>hereditary tumors</u> of the nervous system in addition the eight such diseases already recognized; to add to the clinical description and <u>natural history</u> of these diseases; to suggest methods for <u>early diagnosis</u>; evaluate present modes of <u>treatment</u>; and develop methods for <u>pre-clinical detection</u> and <u>screening</u>.</p> <p>Presently we are evaluating the usefulness of serum <u>nerve growth factor</u> as a screening device for those at risk for <u>central neurofibromatosis</u> and serum <u>norepinephrine</u> as a screening device in the <u>von Hippel-Lindau syndrome</u> and <u>computerized axial tomography</u> as a screening measure in both diseases.</p> <p>Project Number 01930-05 has been incorporated into Project Number Z01 01927-06 ID shown above.</p> | | |

[illegible]

Horton, W.: Genetics of Central Nervous System
Tumors. Birth Defect Series, National Foundation-
March of Dimes, In Press

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| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | | U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | | PROJECT NUMBER Z01 NS 01930 06 ID | |
| PERIOD COVERED July 1, 1975 to June 30, 1976 | | | | | |
| TITLE OF PROJECT (80 characters or less) Von Hippel-Lindau Syndrome: Clinical, Genetic and Biochemical Aspects | | | | | |
| NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT PI: W. Horton UCLA, Los Angeles, CA OTHER: R. Eldridge Head, Section on Neurogenetics ID, NINCDS | | | | | |
| COOPERATING UNITS (if any) Armed Forces Institute of Pathology; Johns Hopkins Hospital, Maryland; Columbia Presbyterian Medical Center, New York; University of Kansas Medical Center, Kansas; Laboratory of Clinical Science, NIMH,NIH | | | | | |
| LAB/BRANCH Infectious Disease Branch | | | | | |
| SECTION Section on Neurogenetics | | | | | |
| INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20014 | | | | | |
| TOTAL MANYEARS: | | PROFESSIONAL: | | OTHER: | |
| SUMMARY OF WORK (200 words or less - underline keywords) This project has been incorporated into Project No. Z01 NS 01927 06 ID. | | | | | |

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|--|--|--|------------|-------------|--------------------------------|------------|--------|-----------|-----------------------|--|--|------------|--------------------|------------|
| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 NS 01977 05 ID | | | | | | | | | | | | |
| PERIOD COVERED <p style="text-align: center;">July 1, 1975 to June 30, 1976</p> | | | | | | | | | | | | | | |
| TITLE OF PROJECT (80 characters or less) <p style="text-align: center;">Selected Genetic and Clinical Aspects of Huntington's Disease</p> | | | | | | | | | | | | | | |
| NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT | | | | | | | | | | | | | | |
| <table style="width: 100%; border: none;"> <tr> <td style="width: 10%;">PI:</td> <td style="width: 30%;">R. Eldridge</td> <td style="width: 40%;">Head, Section on Neurogenetics</td> <td style="width: 20%;">ID, NINCDS</td> </tr> <tr> <td>OTHER:</td> <td>W. Horton</td> <td>UCLA, Los Angeles, CA</td> <td></td> </tr> <tr> <td></td> <td>T. Koerber</td> <td>Research Assistant</td> <td>ID, NINCDS</td> </tr> </table> | | | PI: | R. Eldridge | Head, Section on Neurogenetics | ID, NINCDS | OTHER: | W. Horton | UCLA, Los Angeles, CA | | | T. Koerber | Research Assistant | ID, NINCDS |
| PI: | R. Eldridge | Head, Section on Neurogenetics | ID, NINCDS | | | | | | | | | | | |
| OTHER: | W. Horton | UCLA, Los Angeles, CA | | | | | | | | | | | | |
| | T. Koerber | Research Assistant | ID, NINCDS | | | | | | | | | | | |
| COOPERATING UNITS (if any) <p style="text-align: center;">Committee to Combat Huntington's Disease, NYC, NY</p> | | | | | | | | | | | | | | |
| LAB/BRANCH <p style="text-align: center;">Infectious Disease Branch</p> | | | | | | | | | | | | | | |
| SECTION <p style="text-align: center;">Section on Neurogenetics</p> | | | | | | | | | | | | | | |
| INSTITUTE AND LOCATION <p style="text-align: center;">NINCDS, NIH, Bethesda, Maryland 20014</p> | | | | | | | | | | | | | | |
| TOTAL MANYEARS: | PROFESSIONAL: | OTHER: | | | | | | | | | | | | |
| SUMMARY OF WORK (200 words or less - underline keywords) | | | | | | | | | | | | | | |
| <p>This project has been incorporated into Project No. Z01 NS 01924 06 ID.</p> | | | | | | | | | | | | | | |

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|--|---|--|-------------|-------------|--------------------------------|------------|--------|-----------|-----------------------|--|--|------------|--|-------------|
| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 NS 02046 04 ID | | | | | | | | | | | | |
| PERIOD COVERED July 1, 1975 to June 30, 1976 | | | | | | | | | | | | | | |
| TITLE OF PROJECT (80 characters or less) Clinical, Genetic and Biochemical Study of the Hereditary Epilepsies | | | | | | | | | | | | | | |
| NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT | | | | | | | | | | | | | | |
| <table border="0"> <tr> <td>PI:</td> <td>R. Eldridge</td> <td>Head, Section on Neurogenetics</td> <td>ID, NINCDS</td> </tr> <tr> <td>OTHER:</td> <td>W. Horton</td> <td>UCLA, Los Angeles, CA</td> <td></td> </tr> <tr> <td></td> <td>A. Dekaban</td> <td>Associate Chief, Developmental and Metabolic Neurology Branch</td> <td>DMN, NINCDS</td> </tr> </table> | | | PI: | R. Eldridge | Head, Section on Neurogenetics | ID, NINCDS | OTHER: | W. Horton | UCLA, Los Angeles, CA | | | A. Dekaban | Associate Chief, Developmental and Metabolic Neurology Branch | DMN, NINCDS |
| PI: | R. Eldridge | Head, Section on Neurogenetics | ID, NINCDS | | | | | | | | | | | |
| OTHER: | W. Horton | UCLA, Los Angeles, CA | | | | | | | | | | | | |
| | A. Dekaban | Associate Chief, Developmental and Metabolic Neurology Branch | DMN, NINCDS | | | | | | | | | | | |
| COOPERATING UNITS (if any) Department of Neurology, Columbia University | | | | | | | | | | | | | | |
| LAB/BRANCH Infectious Disease Branch | | | | | | | | | | | | | | |
| SECTION Section on Neurogenetics | | | | | | | | | | | | | | |
| INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20014 | | | | | | | | | | | | | | |
| TOTAL MANYEARS: | PROFESSIONAL: | OTHER: | | | | | | | | | | | | |
| SUMMARY OF WORK (200 words or less - underline keywords) This project has been incorporated into Project No. Z01 NS 01924 06 ID. | | | | | | | | | | | | | | |

Z01 NS 02113 03 ID

PERIOD COVERED

July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Side Effects of L-DOPA on Pre-Pubital Patients with Dystonia

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

| | | | |
|--------|-------------|---|------------|
| PI: | R. Eldridge | Head, Section on Neurogenetics | ID, NINCDS |
| OTHER: | L. Loriaux | Acting Chief, Reproduction Research Branch | RR, NICHD |

COOPERATING UNITS (if any)

None

LAB/BRANCH

Infectious Disease Branch

SECTION

Section on Neurogenetics

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:

PROFESSIONAL:

OTHER:

SUMMARY OF WORK (200 words or less - underline keywords)

This project has been incorporated into Project No. Z01 NS 01924 06 ID.

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| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 NS 02114 03 ID |
| PERIOD COVERED July 1, 1975 to June 30, 1976 | | |
| TITLE OF PROJECT (80 characters or less) The Study of Familial Parkinson's Disease | | |
| NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT PI: W. Horton UCLA, Los Angeles, CA OTHER: R. Eldridge Head, Section on Neurogenetics ID, NINCDS | | |
| COOPERATING UNITS (if any) None | | |
| LAB/BRANCH Infectious Disease Branch | | |
| SECTION Section on Neurogenetics | | |
| INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20014 | | |
| TOTAL MANYEARS: | PROFESSIONAL: | OTHER: |
| SUMMARY OF WORK (200 words or less - underline keywords) This project has been incorporated into Project No. Z01 NS 01924 06 ID. | | |

PERIOD COVERED

July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Familial Amyotrophic Lateral Sclerosis: Clinical, Pathologic and
Genetic StudyNAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER
PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECTPI: W. Horton UCLA, Los Angeles, CA
OTHER: R. Eldridge Head, Section on Neurogenetics ID, NINCDS

COOPERATING UNITS (if any)

Department of Surgery, University of California, CA

LAB/BRANCH

Infectious Disease Branch

SECTION

Section on Neurogenetics

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:

PROFESSIONAL:

OTHER:

SUMMARY OF WORK (200 words or less - underline keywords)

This project has been incorporated into Project No. Z01 NS 02167 02 ID.

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|---|---|--|-------------|-------------|--------------------------------|-------------|--------|--------------|---------------------|------------|--|----------|------------------|-------------|--|----------|----------------------------------|-------------|--|-----------|----------------------------|-------------|--|-----------|-------------------------|-------------|
| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 NS 02167 02 ID | | | | | | | | | | | | | | | | | | | | | | | | |
| PERIOD COVERED July 1, 1975 to June 30, 1976 | | | | | | | | | | | | | | | | | | | | | | | | | | |
| TITLE OF PROJECT (80 characters or less) Immunogenetic Studies in Multiple Sclerosis and Other Neurologic Disorders | | | | | | | | | | | | | | | | | | | | | | | | | | |
| NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT | | | | | | | | | | | | | | | | | | | | | | | | | | |
| <table style="width: 100%; border: none;"> <tr> <td style="width: 10%;">PI:</td> <td style="width: 30%;">R. Eldridge</td> <td style="width: 40%;">Head, Section on Neurogenetics</td> <td style="width: 20%;">IDB, NINCDS</td> </tr> <tr> <td>OTHER:</td> <td>H. McFarland</td> <td>Senior Investigator</td> <td>NI, NINCDS</td> </tr> <tr> <td></td> <td>H. Krebs</td> <td>Nurse Specialist</td> <td>IDB, NINCDS</td> </tr> <tr> <td></td> <td>J. Sever</td> <td>Chief, Infectious Disease Branch</td> <td>IDB, NINCDS</td> </tr> <tr> <td></td> <td>D. Madden</td> <td>Head, Unit on Microbiology</td> <td>IDB, NINCDS</td> </tr> <tr> <td></td> <td>M. Gravel</td> <td>Research Microbiologist</td> <td>IDB, NINCDS</td> </tr> </table> | | | PI: | R. Eldridge | Head, Section on Neurogenetics | IDB, NINCDS | OTHER: | H. McFarland | Senior Investigator | NI, NINCDS | | H. Krebs | Nurse Specialist | IDB, NINCDS | | J. Sever | Chief, Infectious Disease Branch | IDB, NINCDS | | D. Madden | Head, Unit on Microbiology | IDB, NINCDS | | M. Gravel | Research Microbiologist | IDB, NINCDS |
| PI: | R. Eldridge | Head, Section on Neurogenetics | IDB, NINCDS | | | | | | | | | | | | | | | | | | | | | | | |
| OTHER: | H. McFarland | Senior Investigator | NI, NINCDS | | | | | | | | | | | | | | | | | | | | | | | |
| | H. Krebs | Nurse Specialist | IDB, NINCDS | | | | | | | | | | | | | | | | | | | | | | | |
| | J. Sever | Chief, Infectious Disease Branch | IDB, NINCDS | | | | | | | | | | | | | | | | | | | | | | | |
| | D. Madden | Head, Unit on Microbiology | IDB, NINCDS | | | | | | | | | | | | | | | | | | | | | | | |
| | M. Gravel | Research Microbiologist | IDB, NINCDS | | | | | | | | | | | | | | | | | | | | | | | |
| COOPERATING UNITS (if any) Department of Surgery, University of California; Department of Medical Genetics, University of Indianapolis Medical Center | | | | | | | | | | | | | | | | | | | | | | | | | | |
| LAB/BRANCH Infectious Disease Branch | | | | | | | | | | | | | | | | | | | | | | | | | | |
| SECTION Section on Neurogenetics | | | | | | | | | | | | | | | | | | | | | | | | | | |
| INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20014 | | | | | | | | | | | | | | | | | | | | | | | | | | |
| TOTAL MANYEARS: 1.50 | PROFESSIONAL: .50 | OTHER: 1.0 | | | | | | | | | | | | | | | | | | | | | | | | |
| SUMMARY OF WORK (200 words or less - underline keywords) | | | | | | | | | | | | | | | | | | | | | | | | | | |
| <p> In this project we are evaluating the role of <u>genetic factors</u> in diseases due apparently to disordered <u>immunity</u>. These diseases include <u>multiple sclerosis</u>, <u>Reye's syndrome</u> and <u>amyotrophic lateral sclerosis</u>. In addition to clarifying etiologic mechanisms such an approach may indicate individuals or <u>populations at high risk</u> and suggest mechanisms for <u>prevention</u> and <u>treatment</u>. </p> <p> We are making use of standard <u>epidemiologic</u> and genetic techniques as well as promising immunogenetically based laboratory approaches. In the case of multiple sclerosis, such investigation consists of: comprehensive study of the <u>histocompatibility complex</u> with determination of <u>HLA</u>, <u>MLC</u> and <u>B lymphocyte</u> genotypes; <u>linkage</u> studies for serum proteins; viral <u>serology</u>; and the search for the "<u>Carp</u>" agent. </p> <p> Project Number 02115 02 has been incorporated into Project Number NS 02167-02 ID shown above. </p> | | | | | | | | | | | | | | | | | | | | | | | | | | |

Project Description:

Objectives: Recent strides in knowledge regarding the genetic control of the immune response have suggested new avenues for exploring diseases such as multiple sclerosis which may be due to disordered immunity. It is the purpose of this project to evaluate genetic factors in multiple sclerosis, amyotrophic lateral sclerosis, Reye's syndrome and other conditions which may be due to unusual immunologic reaction to common infectious agents. Such an approach may clarify etiologic mechanisms in these disease states, indicate high risk individuals and populations, and suggest possible mechanisms of prevention and treatment.

Methods Employed: Classical techniques of clinical genetic study will be utilized. Carefully selected populations including families with multiple members affected and identical twin pairs discordant or concordant for the disease in question will be studied in depth. Unaffected family members and unaffected twins will serve as controls. Studies will consist of careful medical and epidemiologic history, neurologic exam, genealogic history and appropriate immunogenetically based laboratory investigation. The latter, in the case of multiple sclerosis will consist of: comprehensive study of the histocompatibility complex including determination of HLA, MLC and B lymphocyte genotypes; standard serum protein genotyping with particular emphasis on those proteins whose genes may reside near the histocompatibility complex; serologic evaluation of exposure to measles and other viruses; and study of the "Carp" agent utilizing mouse and PAM cell systems.

Major Findings: In the initial phase of the familial multiple sclerosis study 100 individuals in 12 kindreds have been evaluated. Although the laboratory data has not been fully processed as yet one finding is already apparent: unlike two reports in the very recent neurologic literature suggesting "linkage" between the gene or genes predisposing to multiple sclerosis and the histocompatibility complex of chromosome six, we find no evidence to support such linkage.

Significance to Biomedical Research and the Program of the Institute: Disorders due to possible abnormality in the immune response, such as multiple sclerosis and amyotrophic lateral sclerosis, comprise a major neurologic public health problem. Ample evidence from family studies and population data already indicates genetic factors have a role in the causation of multiple sclerosis. By coupling existing knowledge of genetics with that of the immune response emerging from various laboratories understanding of this group of disorders should be advanced.

Proposed Course: Results of the first phase of the familial multiple sclerosis study are to be reported at the "First International Symposium on HLA" in late June, 1976.

Another phase of the study will involve immunogenetic study of identical twins with multiple sclerosis. Approximately 15 twin pairs have already been ascertained and considerably more are anticipated in response to a

forthcoming announcement by the National Multiple Sclerosis Society indicating out interest. Because of the small, selected nature of these multiple sclerosis populations under study it is generally possible for us to incorporate the newest developments in the field of immunogenetics.

Publications: Horton, W., and Eldridge, R.: Familial motor neuron disease: Evidence for at least three different types.
Neurology, In Press

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|--|---|--|------------|-------------|--------------------------------|------------|--------|------------|--------------------|-----------|--|-----------|--------------------|-----------|
| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 NS 02168 02 ID | | | | | | | | | | | | |
| PERIOD COVERED July 1, 1975 to June 30, 1976 | | | | | | | | | | | | | | |
| TITLE OF PROJECT (80 characters or less) Clinical, Genetic and Biochemical Observations in Families with Gilles de la Tourette Syndrome (GTS) | | | | | | | | | | | | | | |
| NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT <table style="width: 100%; border: none;"> <tr> <td style="width: 10%;">PI:</td> <td style="width: 30%;">R. Eldridge</td> <td style="width: 40%;">Head, Section on Neurogenetics</td> <td style="width: 20%;">ID, NINCDS</td> </tr> <tr> <td>OTHER:</td> <td>M. Ziegler</td> <td>Research Associate</td> <td>LCS, NIMH</td> </tr> <tr> <td></td> <td>C.R. Lake</td> <td>Research Associate</td> <td>LCS, NIMH</td> </tr> </table> | | | PI: | R. Eldridge | Head, Section on Neurogenetics | ID, NINCDS | OTHER: | M. Ziegler | Research Associate | LCS, NIMH | | C.R. Lake | Research Associate | LCS, NIMH |
| PI: | R. Eldridge | Head, Section on Neurogenetics | ID, NINCDS | | | | | | | | | | | |
| OTHER: | M. Ziegler | Research Associate | LCS, NIMH | | | | | | | | | | | |
| | C.R. Lake | Research Associate | LCS, NIMH | | | | | | | | | | | |
| COOPERATING UNITS (if any) Cornell Medical School, New York University; Department of Medicine, University of California; Department of Pharmacology, Mt. Sinai Hospital | | | | | | | | | | | | | | |
| LAB/BRANCH <u>Infectious Disease Branch</u> | | | | | | | | | | | | | | |
| SECTION <u>Section on Neurogenetics</u> | | | | | | | | | | | | | | |
| INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20014 | | | | | | | | | | | | | | |
| TOTAL MANYEARS: | PROFESSIONAL: | OTHER: | | | | | | | | | | | | |
| SUMMARY OF WORK (200 words or less - underline keywords) <p style="text-align: center;">This project has been incorporated into Project No. Z01 01924 06 ID.</p> | | | | | | | | | | | | | | |

Annual Report
July 1, 1975 to June 30, 1976
Neuroimmunology Branch
National Institute of Neurological and
Communicative Disorders and Stroke

Dale E. McFarlin, M.D., Chief

The Neuroimmunology Branch officially established last year devoted major efforts toward becoming an effective scientific group. By February 1, 1976 the laboratory space initially designated for use by this Branch had been acquired and renovated; this is now functional. At various times over the past year, nine of the personnel arrived and began laboratory and clinical investigations. An outpatient clinic for evaluation of patients with multiple sclerosis and other diseases having immunological abnormalities has been established. Beginning January 1976, inpatients with multiple sclerosis were admitted to the clinical center for study. Two animal models, experimental allergic encephalomyelitis (EAE) and murine measles infection are being studied.

The investigations of NI fall into four areas:

(1) The interaction between virus and the host immune response. The major objective of these investigations is to determine the mechanisms by which interaction between virus and the host immune system either facilitates or inhibits the development of acute and/or chronic viral infections. The effect of viral infections upon specific T-cell and B-cell functions is being studied. It is anticipated that a possible relationship between the host histocompatibility background and the infected cells will be studied.

(2) The organization of the myelin membrane. Because disease states involving the central nervous system may be related to an immunological attack on myelin, elucidation of the molecular organization is important. This work is designed to further identify the chemical composition of myelin, particularly with respect to the outer membrane surface, which could be more readily susceptible to immunological damage and to viral infection. The results so far indicate that a major molecular component of the outer membrane surface of myelin is a glycoprotein. The possible role of this glycoprotein in both natural and experimental demyelinating disease can be examined with appropriate immunological studies. Further, since glycoproteins have been found to play an important role in cell recognition, it will be possible to study the relationship of the major myelin

glycoproteins to those associated with other cell membranes such as the lymphocyte. Similar approaches will be taken to study the lipid components of the myelin membrane.

(3) Effector mechanisms operative in EAE. The production of this disease is most likely related to a cell-mediated immune response to a CNS antigen, basic protein. However, the actual cellular effector mechanisms are unknown. This component is being studied by examining the specific roles of T cells with respect to their ability to transfer the disease as well as their ability to recognize and respond to basic protein in vitro.

In addition, EAE is being studied in the mouse and rat with respect to the influence of genetic factors on disease expression. This includes in vitro studies of the genetic influence on T cell recognition and responsiveness to basic protein as well as studies of disease susceptibility in various strains.

(4) Assessment of the immune function in patients with multiple sclerosis. In collaboration with the Neurology Department of Johns Hopkins University and the IDB of IRP, a series of patients with "pal" controls have been investigated with respect to histocompatibility background. The results confirm an increased representation of HL-A3 and HL-B7 and the HL-DW2. The latter antigen is present in approximately 70% of the patients with multiple sclerosis and occurs in approximately 16% of control individuals. The serum antibody titers against a number of viral agents are being determined.

Spinal fluid is being studied for immunoglobulin content and antibody against viruses. A highly sensitive radioimmunoassay for the quantitation of CSF IgG, IgA and IgM has been developed. This assay has demonstrated that CSF IgG is elevated in the majority of the patients with active multiple sclerosis which confirms results obtained by rocket immunoelectrodifusion. In addition, this assay is demonstrating an increase in CSF IgM and IgA in some patients with multiple sclerosis. Further efforts will be made to relate such findings to the clinical stage of this disease and to seek the antigen(s) against which these immunoglobulins are directed. A collaborative immunopathological study on specimens from MS patients has been initiated with the New Jersey Medical School and the Albert Einstein Medical School. Investigation of families with more than one occurrence of multiple sclerosis using a combination of serological, immunological, and genetic techniques has been initiated in collaboration with IDB.

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| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 NS 02202-01 NI |
| PERIOD COVERED <p style="text-align: center;">July 1, 1975 to June 30, 1976</p> | | |
| TITLE OF PROJECT (80 characters or less) <p style="text-align: center;">Immunological Studies in Patients with Multiple Sclerosis and Other CNS Diseases.</p> | | |
| NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT | | |
| PI: | D. E. McFarlin H. F. McFarland | Chief Asst. Chief |
| OTHER: | L. B. Barnett J. L. Sever R. Eldridge | NI NINCDS NI NINCDS NI NINCDS ID NINCDS ID NINCDS |
| COOPERATING UNITS (if any) <p style="text-align: center;">Department of Neurology, Johns Hopkins Hospital, Baltimore, Md.</p> | | |
| LAB/BRANCH <p style="text-align: center;">Neuroimmunology</p> | | |
| SECTION <p style="text-align: center;">Office of the Chief</p> | | |
| INSTITUTE AND LOCATION <p style="text-align: center;">NINCDS, NIH, Bethesda, Maryland 20014</p> | | |
| TOTAL MANYEARS: <p style="text-align: center;">4.0</p> | PROFESSIONAL: <p style="text-align: center;">2.0</p> | OTHER: <p style="text-align: center;">2.0</p> |
| SUMMARY OF WORK (200 words or less - underline keywords) <p> The general aim of this project is to obtain a more precise understanding of the role of genetic factors in multiple sclerosis. This will include a number of interrelated questions which will include; (1) determination of <u>histocompatibility</u> types in a carefully selected population of MS patients and appropriate control individuals. (2) Correlation of histocompatibility data in both the patients and controls to the humoral and cell-mediated immune response to measles virus. (3) Evaluation of <u>cerebrospinal fluid</u> immunoglobulin content and specificity in <u>multiple sclerosis</u> patients. (4) Evaluation of families with a multiple incidence in multiple sclerosis and examination of affected and nonaffected members of these families with respect to both histocompatibility makeup and immunological response. (5) Identification of additional lymphocyte antigens which may show a greater degree of correlation with multiple sclerosis than presently identified lymphocyte antigens. </p> | | |

Project Description:

Objective: The goal of this project is to obtain a more precise understanding of the role of genetic factors in multiple sclerosis (MS). This will include studies of the relationship of histocompatibility linked genes to immunological responsiveness to viral antigens in MS patients and control individuals. Numerous interrelated questions will be examined and these include:

1. Examination of the histocompatibility make up in a population of patients who have been carefully evaluated clinically. Findings in these patients will be compared to "pal" and sibling controls.
2. Correlation of histocompatibility composition with the humoral and cell-mediated immune response to measles virus. This will include evaluation of various assays of cell-mediated immunity to viruses and examination of the possible need for histocompatibility similarity between effector and target cells in the various assays.
3. Evaluation of cerebrospinal fluid (CSF) immunoglobulin content in MS patients and correlation with histocompatibility type. This will include studies of the total IgG, IgA and IgM content as well as anti measles titer.
4. Evaluation of families with a multiple incidence of MS and examination of affected and unaffected family members with respect to histocompatibility antigens and humoral and cell-mediated immune response to measles virus.
5. Studies of possible lymphocyte antigens which may have a greater degree of correlation with MS than current SD or LD antigens. This will utilize serum and peripheral blood lymphocytes obtained from families of MS patients. If additional lymphocyte markers are detected, they will be correlated with immunological function as described in (2) above.

Methods Employed:

General - The clinical populations consists of patients and controls from the MS clinic of Johns Hopkins Hospital and patients seen in MS clinic at NIH. In each case the patients are characterized clinically, and histocompatibility typing is being performed under contract by Dr. Paul Terasaki. Serological and immunological studies are being conducted in the IDB and NIB.

Serology - Conventional antibody assays will be performed on serum and CSF when available. In addition, radioimmunoassays will be employed in the CSF to determine antibody against measles virus and the composition of immunoglobulins.

Cell-mediated immunity - Assays of lymphocyte transformation and cytotoxicity will be performed using peripheral blood lymphocytes. Target cells will consist of both syngenic and homogenic responses to both cell-associated virus and purified viral antigens will be measured.

Lymphocyte antigens - Sera from multiporous wives and mothers of MS patients will be utilized in assays for antigens on T and B lymphocytes in MS patients. Appropriate absorptions will be performed to define the relationship of these antigens to known SD and LD antigens.

Major Findings:

Histocompatibility studies in MS.

In the 30 MS patients and 50 controls now studied an increased incidence of HLA-B7 and HLA-DW2 has been observed in the MS populations.

Correlation of histocompatibility with antiviral antibodies.

A significant increase in CF and HI antimeasles titers has been found in the MS group in comparison to the control group. Only a weak correlation has been found between HLA-B7 and antimeasles titers. There were no significant differences in titers to Herpes Simples, Rubella, or Vaccinia Viruses.

CSF immunoglobulins.

A sensitive radioimmunoassay has been developed for the detection of CSF IgG, IgA and IgM. This method permits quantitation of CSF immunoglobulins without concentration and is a more sensitive tool than immunoelectrodiffusion.

Lymphocyte antigens.

The radioimmunoassay for cell membrane antigens employing staphylococcus protein A has been developed; this method appears efficacious for detecting small amounts of cell-bound antibody.

Family studies.

Five families in Wisconsin and 5 families in Kansas have been evaluated clinically. In addition two families have been identified and studied in the Washington area. The serologic, genetic and immunologic studies described above will be conducted.

Publications:

McFarlin, D. E. and McFarland H.F.: Histocompatibility studies and multiple sclerosis. Arch. Neurol. (in press).

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| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 NS 02203-01 NI | | | | | | | | | | | | | | | | | | | | |
| PERIOD COVERED <p style="text-align: center;">July 1, 1975 to June 30, 1976</p> | | | | | | | | | | | | | | | | | | | | | | |
| TITLE OF PROJECT (80 characters or less) <p style="text-align: center;">Membrane Structure and Function in the Nervous System.</p> | | | | | | | | | | | | | | | | | | | | | | |
| NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT | | | | | | | | | | | | | | | | | | | | | | |
| <table style="width: 100%; border: none;"> <tr> <td style="width: 15%; vertical-align: top;">PI:</td> <td style="width: 35%;">J. F. Poduslo</td> <td style="width: 20%;">Staff Fellow</td> <td style="width: 30%;">NI NINCDS</td> </tr> <tr> <td style="vertical-align: top;">OTHER:</td> <td>R. B. Brady</td> <td>Chief</td> <td>DMN NINCDS</td> </tr> <tr> <td></td> <td>R. H. Quarles</td> <td>Res. Assoc.</td> <td>DMN NINCDS</td> </tr> <tr> <td></td> <td>D. E. McFarlin</td> <td>Chief</td> <td>NI NINCDS</td> </tr> <tr> <td></td> <td>H. F. McFarland</td> <td>Asst. Chief</td> <td>NI NINCDS</td> </tr> </table> | | | PI: | J. F. Poduslo | Staff Fellow | NI NINCDS | OTHER: | R. B. Brady | Chief | DMN NINCDS | | R. H. Quarles | Res. Assoc. | DMN NINCDS | | D. E. McFarlin | Chief | NI NINCDS | | H. F. McFarland | Asst. Chief | NI NINCDS |
| PI: | J. F. Poduslo | Staff Fellow | NI NINCDS | | | | | | | | | | | | | | | | | | | |
| OTHER: | R. B. Brady | Chief | DMN NINCDS | | | | | | | | | | | | | | | | | | | |
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| | D. E. McFarlin | Chief | NI NINCDS | | | | | | | | | | | | | | | | | | | |
| | H. F. McFarland | Asst. Chief | NI NINCDS | | | | | | | | | | | | | | | | | | | |
| COOPERATING UNITS (if any) | | | | | | | | | | | | | | | | | | | | | | |
| LAB/BRANCH Neuroimmunology | | | | | | | | | | | | | | | | | | | | | | |
| SECTION Office of the Chief | | | | | | | | | | | | | | | | | | | | | | |
| INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20014 | | | | | | | | | | | | | | | | | | | | | | |
| TOTAL MANYEARS: <p style="text-align: center;">1.5</p> | PROFESSIONAL: <p style="text-align: center;">1.0</p> | OTHER: <p style="text-align: center;">0.5</p> | | | | | | | | | | | | | | | | | | | | |
| SUMMARY OF WORK (200 words or less - underline keywords) | | | | | | | | | | | | | | | | | | | | | | |
| <p> The goal of this project is to characterize the chemical composition of the <u>myelin</u> membrane in the central nervous system. This study has focused on the composition of the outer membrane surface. Using a tritium label with an enzymatic membrane probe a major myelin <u>glycoprotein</u> has been identified on the outer membrane surface of myelin. Studies of the myelin <u>glycolipids</u> have also revealed a asymmetric distribution of the <u>glycolipids</u> in the myelin sheath. </p> | | | | | | | | | | | | | | | | | | | | | | |

Project Description:

Objective: This project is designed to obtain information concerning the molecular organization of myelin in the central nervous system. Specifically, the investigation is concerned with the topographical localization of individual myelin components with respect to the outer membrane surface or the inner, cytoplasmic membrane surface. The rationale for such a study is that the identification of surface constituents on the intact myelin sheath will not only increase our understanding of the molecular organization of myelin, but will also facilitate an understanding of their potential functional properties in the membrane. Such surface membrane components may not only play important recognition roles in the process of myelination or myelin maintenance but may also have potential antigenic properties important for the generation of immune tolerance or a specific autoimmune response as a consequence of a disease process.

Experimental Approach: An increasing body of experimental evidence indicates that most biological membranes have an asymmetrical distribution of their components. Furthermore, those components that are localized on the external surface of the membrane have been found to be predominantly carbohydrate containing proteins or carbohydrate containing lipids. Therefore, it was rationalized that the identification of myelin surface constituents might be facilitated by employing a membrane probe with a high degree of specificity for carbohydrate residues. Consequently, the enzymatic membrane probe, galactose oxidase, was selected for the covalent labeling of surface membrane glycoproteins and glycolipids. Galactose oxidase specifically oxidizes D-galactose and related sugars at the carbon-6 position with the formation of D-galactohexodialdose. The resulting aldehyde group can then be labeled by reduction back to the primary hydroxyl group with tritiated sodium borohydride. Since galactose oxidase is impermeant to the membrane, only those sugar residues that are accessible to the enzyme will be oxidized.

Methods Employed: Excised spinal cords from Osborne-Mendel rats were stripped off their meninges and placed in a specially designed incubation chamber. The ends of the spinal cord were sealed with petroleum jelly and the enzymatic reaction was performed directly in this incubation chamber followed by brief treatment with the tritiated reducing agent. After incorporation of tritium into the modified glycoproteins and glycolipids, myelin was isolated and

either analyzed electrophoretically for the amount of tritium associated with individual protein bands or partitioned with chloroform-methanol-water and analyzed by thin layer chromatography for the tritium associated with individual glycolipids.

Major Findings:

Results indicated tritium label associated with a surprising variety of high molecular weight proteins. The most extensively labeled peak corresponded to the major myelin glycoprotein as indicated by the coincidence of tritium label with that of [^{14}C] fucose used as an internal marker for the glycoproteins. The radioactivity associated with this protein was 1.1 to 2.7 times higher after treatment with galactose oxidase when compared to reduction in the absence of the enzyme and 1.4 to 4.8 times higher when oxidized and reduced after prior treatment with neuraminidase. The results suggest a complex heterogeneity of minor glycoproteins associated with isolated myelin. It is concluded that from this complexity of glycoproteins, a major glycoprotein is at least partially localized on the external surface of either the intact myelin sheath or the closely associated oligodendroglial plasma membrane. Such a localization of this glycoprotein and the probable localization of the other glycoproteins enhances their potential role in specific interactions in the process of myelination or myelin maintenance.

Analysis of the myelin glycolipids also revealed an asymmetrical distribution of glycolipids in the myelin sheath. Results indicated extensive non-specific reduction of the normal fatty acid galactocerebroside which accounted for 40-50% of the recovered counts. In contrast, the 2-hydroxyl fatty acid equivalent accounted for only 4% of the counts whereas sulfatide accounted for 10-12%. Treatment with galactose oxidase resulted in 1.6 to 1.9 fold increase of label in only the nonhydroxyl fatty acid galactocerebroside. Confirmation of the specificity of the reaction was obtained by showing label associated with galactose after acid hydrolysis of this isolated glycolipid. The specific labeling of this particular glycolipid as compared to other myelin lipids implies an external membrane location on the myelin sheath and lends credence to the notion that cerebroside may play a role as a myelin surface antigen.

Publications:

Poduslo, J.F., Quarles, R.H., and Brady, R.O.: External labeling of galactose in surface membrane glycoproteins of the intact myelin sheath. J. Biol. Chem. 251: 153-158, 1975.

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| PERIOD COVERED <p style="text-align: center;">July 1, 1975 to June 30, 1976</p> | | | | | | | | | | | | | | | | | | |
| TITLE OF PROJECT (80 characters or less) <p style="text-align: center;">Immunologic Mechanisms Operative in Experimental Allergic Encephalomyelitis (EAE).</p> | | | | | | | | | | | | | | | | | | |
| NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT | | | | | | | | | | | | | | | | | | |
| <table style="width: 100%; border: none;"> <tr> <td style="width: 15%;">PI:</td> <td style="width: 35%;">H. S. Panitch</td> <td style="width: 20%;">Staff Fellow</td> <td style="width: 30%;">NI NINCDS</td> </tr> <tr> <td>OTHER:</td> <td>D. E. McFarlin</td> <td>Chief</td> <td>NI NINCDS</td> </tr> <tr> <td></td> <td>L. B. Barnett</td> <td>Staff Fellow</td> <td>NI NINCDS</td> </tr> <tr> <td></td> <td>J. F. Poduslo</td> <td>Staff Fellow</td> <td>NI NINCDS</td> </tr> </table> | | | PI: | H. S. Panitch | Staff Fellow | NI NINCDS | OTHER: | D. E. McFarlin | Chief | NI NINCDS | | L. B. Barnett | Staff Fellow | NI NINCDS | | J. F. Poduslo | Staff Fellow | NI NINCDS |
| PI: | H. S. Panitch | Staff Fellow | NI NINCDS | | | | | | | | | | | | | | | |
| OTHER: | D. E. McFarlin | Chief | NI NINCDS | | | | | | | | | | | | | | | |
| | L. B. Barnett | Staff Fellow | NI NINCDS | | | | | | | | | | | | | | | |
| | J. F. Poduslo | Staff Fellow | NI NINCDS | | | | | | | | | | | | | | | |
| COOPERATING UNITS (if any) <p style="text-align: center;">Department of Neurology, Emory University, Atlanta, Georgia</p> | | | | | | | | | | | | | | | | | | |
| LAB/BRANCH <p style="text-align: center;">Neuroimmunology</p> | | | | | | | | | | | | | | | | | | |
| SECTION <p style="text-align: center;">Office of the Chief</p> | | | | | | | | | | | | | | | | | | |
| INSTITUTE AND LOCATION <p style="text-align: center;">NINCDS, NIH, Bethesda, Maryland 20014</p> | | | | | | | | | | | | | | | | | | |
| TOTAL MANYEARS: <p style="text-align: center;">2.0</p> | PROFESSIONAL: <p style="text-align: center;">1.5</p> | OTHER: <p style="text-align: center;">0.5</p> | | | | | | | | | | | | | | | | |
| SUMMARY OF WORK (200 words or less - underline keywords) | | | | | | | | | | | | | | | | | | |
| <p> The aim of this project is to identify the relative role of various lymphocyte populations in disease production in <u>experimental allergic encephalomyelitis</u>, a model of autoimmune disease. These studies will focus on the ability to transfer EAE with various populations of lymphocytes particularly <u>T</u> and <u>B cell</u> populations. In addition the <u>in vitro</u> response of these cell populations to <u>myelin basic protein</u> will be examined. The relationship of the host genetic makeup to disease susceptibility will also be examined both <u>in vitro</u> and <u>in vivo</u>. The <u>in vitro</u> studies will consist of <u>T cell</u> recognition and responsiveness to <u>basic protein</u>. <u>In vivo</u> studies will include studies of the differences in disease production in various strains of both mice and rats. </p> | | | | | | | | | | | | | | | | | | |

Project Description:

Objective: This project is designed to determine the role of specific populations of immune lymphoid cells in EAE, a model autoimmune disease of the CNS which bears a number of clinical, histological, and immunological features similar to human demyelinating diseases and chronic encephalitis.

The following aspects are being investigated in susceptible Lewis rats:

- a) The identification of T and B lymphocytes.
- b) The in vitro reactivity of lymphoid cell populations to the encephalitogenic antigen, the basic protein (BP) of myelin.
- c) The capacity of purified cell populations to transfer EAE to normal recipients.
- d) In addition, the immune response to basic protein is being studied in various strains of mice and rats with different genetic backgrounds in order to elucidate the relationship of histocompatibility make up to disease mechanisms.

Methods Employed:

a) Preparation of antisera for demonstration of lymphocyte surface antigens. Alloantisera (anti θ , anti Thy 1.1) were prepared in mice, and heterologous sera were prepared in rabbits against rat brain, thymocytes, and lymph node cells (LNC). These were tested for reactivity against rat cells by immunofluorescence (FA) and cytotoxicity.

b) Lymphocyte stimulation (LS) with mitogens and GPBP. Optimal culture conditions have been defined for these assays which have been performed both alone and in conjunction with column separations and transfer experiments described below. LS responses of cells treated with specific antisera and complement (C) are in progress.

c) Separation of rat T and B lymphocytes on nylon wool columns and anti rat IgG coupled to Sephadex, a solid phase immunoabsorbent column. The lymphoid cells are separated by these methods and characterized by FA, cytotoxicity, and lymphocyte stimulation as measured by thymidine incorporation.

d) Transfer of EAE with sensitized LNC. Both LNC preparations and column-purified T cells are being evaluated for their ability to transfer EAE. Dose response curves are performed. Clinical and histological grading as well as determination of anti BP antibody by radioimmunoassay (RIA) are in serum from the recipients.

e) EAE in the mouse. Baseline studies have been initiated in inbred strains of mice which are challenged with rat basic protein in various adjuvants. Clinical disease, CNS pathology, cell-mediated immunity and antibody formation are being evaluated.

Major Findings:Demonstration of lymphocyte surface antigens by antisera:

Cross-reactivity of mouse anti Thy 1.1, rabbit anti mouse brain, and rabbit anti rat brain sera against rat thymocytes was demonstrated. However, these sera had little activity against rat peripheral (spleen or lymph node) T cells in FA or cytotoxicity studies utilizing both trypan blue uptake and ^{51}Cr release. Various sources of C (guinea pig, rabbit, rat) gave similar results. Incubation of cells for 24 or 48 hrs in the presence of PHA had no effect on expression of T cell surface antigens. Other antisera being evaluated are rabbit anti rat thymocyte serum absorbed with rat RBC, fetal liver, and bone marrow; and rabbit anti rat LNC serum prepared by inoculation of the T cell fraction from nylon wool columns. The development of an antiserum specific for rat peripheral T cells is necessary for evaluation of the role of these cells in the development and transfer of EAE, and in relation to the functions of subpopulations of T cells as defined by other measures.

In vitro studies utilizing LS:

Optimal culture conditions which minimize background and maximize responses to mitogens and GPBP established that the use of normal rat serum and low concentrations of 2-mercapto-ethanol (2-ME) are required. Cells passed through nylon wool columns tended to lose viability in culture; the addition of 2-ME restored mitogen responses and permitted demonstration of an augmented response to GPBP (up to 20-fold stimulation) in the T cell fractions. Similar results were obtained with Sephadex cellular adsorbent columns, and in addition the response of the B cell fraction was markedly reduced; this produces other evidence that B cells do not participate in the in vitro LS by BP.

Column separation of cells:

Cells purified by the above methods were characterized by staining with a rabbit anti rat IgG fluorescent conjugate, and by LS responses to T and B cell mitogens and to GPBP. Separated T cells were essentially as effective as whole LNC in transferring EAE to normal recipients.

Transfer experiments:

Optimal transfer of clinical and histological EAE was obtained with 5×10^7 LNC per rat. The transfer of lower numbers of cells (1×10^8) produced milder diseases. Following adrenalectomy rats were more sensitive to low doses of cells; such amounts are being used to assay purified cell fractions. LS responses to BP corresponded with ability of cells to transfer EAE. Antibody formation in donor rats and in recipients of either whole LNC or T cell fractions is being determined.

EAE in the mouse:

Since recent reports have demonstrated that EAE may be induced in this species, studies were undertaken to characterize in vivo and in vitro responses to BP of various inbred strains. Preliminary results suggest that SJL mice sensitized with RBP develop both cell-mediated and humoral responses to the antigen, with increased lymph stimulation and elevated antibody titers by RIA compared to Balb/c mice which appear to be genetically unresponsive.

Publications:

McFarlin, D.E., Hsu, S.C-L., Slemenda, S.B., Chou, S.C-H., and Kibler, R.F.: The immune response against an encephalitogenic fragment of guinea pig basic protein in the Lewis and Brown Norway strains of rat. J. Immunol. 115: 1456-1458, 1975.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE
PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF
HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02205-01 NI

PERIOD COVERED

July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Interaction Between Viruses and Host Immune-System.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER
PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

| | | | |
|--------|-----------------|--------------|------------|
| PI: | E. D. Johnson | Staff Fellow | NI NINCDS |
| OTHER: | H. F. McFarland | Asst. Chief | NI NINCDS |
| | D. E. McFarlin | Chief | NI NINCDS |
| | H. C. Morse | Res. Assoc. | LMI, NIAID |
| | R. Asofsky | Chief | LMI, NIAID |

COOPERATING UNITS (if any)

LAB/BRANCH

Neuroimmunology

SECTION

Office of the Chief

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:

1.5

PROFESSIONAL:

1.0

OTHER:

0.5

SUMMARY OF WORK (200 words or less - underline keywords)

The purpose of this study is to examine the role of the host immune response on both acute and chronic viral infections of the central nervous system. These studies will examine the host immune response with relationship to mechanisms of protection as well as disease production in a viral infected host. In addition, the effect of virus on the host immune response will be examined. Specifically, this will include the effect of virus on the functional capacity of both T and B lymphocytes.

Project Description:

Objective: The major objective of this project is to determine the mechanism(s) by which the interaction between virus and host immune system may facilitate or inhibit the development of acute and/or chronic viral infections of the central nervous system (CNS). In so doing two areas will be studied: (1) the mechanism(s) by which viral infections may lead to dysfunction of the host immune system; (2) the mechanism(s) by which the host humoral and/or cellular virus-specific immune response clears virus from an infected animal.

Methods Employed:Virus-induced immunosuppression -

The ability to develop both a cellular and humoral immune response has been shown to be controlled by the cooperative interaction between functionally heterogeneous thymus-derived and bone marrow-derived lymphocytes. The mechanism by which this interaction takes place has been demonstrated both in vitro and in vivo by the utilization of thymus-dependent and thymus-independent antigens. Therefore, the ability of the host to respond to these antigens during measles virus infections is being studied.

Virus-specific immune response -

Utilizing radioactive label release assays, the virus-specific antibody and cytolytically active lymphocytes capable of destroying virus infected cells in vitro are being studied. In addition, the presences of lymphocytes reactive to virus is being sought by blast transformation, as measured by uptake of radioactive label, in the spleens of animals inoculated with virus. Employing these techniques, the ability of mice to respond to viral antigens will be studied.

Major Findings:

The above studies have just recently been initiated. No findings are yet available.

Publications:

None

ANNUAL REPORT

July 1, 1975 - June 30, 1976

Neurological Disorders Program

National Institute of Neurological and Communicative Disorders and Stroke
National Institutes of Health

ADMINISTRATIVE

The reorganization of the National Institute of Neurological and Communicative Disorders and Stroke was announced in the Federal Register on May 25, 1975 and implementation began on July 15, 1975 with the establishment of the Office of the Director of the Neurological Disorders Program and the other three substantive program areas. Soon thereafter, an Administrative Officer and his staff were appointed for the Neurological Disorders Program and permanent space assignments were made in the basement, 1st floor, 7th floor and 8th floor of the Federal Building and laboratory space on campus in Building 36. Although the space loading of the Federal Building is within GSA criteria, the configuration of this space makes optimal utilization impossible. In addition, renovations which were requested two years ago for our video, electronic, and computer areas have not been completed, making it difficult to adequately perform our functions in these areas. Much of our efforts from July 1975 through December 1975 were directed toward moves, renovations, and organization under the reorganization plan. During August 1975 NIH approved the formation of an Epilepsy Branch and a Developmental Neurology Branch within the Neurological Disorders Program. In September 1975, a Neurological Disorders Program employee meeting was held in order to brief all of our staff on the new Institute and Program structure, the NDP mission and our role within NINCDS. In October, a Health Science Administrator was appointed for NDP and he joined us on the 7th floor. In January, a second Health Science Administrator joined NDP and in April our third Health Science Administrator was recruited and reported for duty in May. This Health Science Administration Unit is functioning within the Office of the Director with a backup staff of three clerical employees. During January and February of 1976, all 60,000 of the collaborative perinatal project case files were moved from the 9th floor of the Federal Building to renovated space in the basement of the Federal Building. This was a massive undertaking; our permanent and temporary employees did a commendable job and were rewarded with a group award for outstanding service. Not a single case file was lost or misplaced during this move. At the present time, we are recruiting specialists in multiple sclerosis and infections of the nervous system; autism and behavioral disorders; Parkinson's Disease, dementia and degenerative diseases of the nervous system; a Deputy Chief for the Epilepsy Branch; and a Deputy Director for the Office of the Director of the Neurological Disorders Program. Although many of our grant procedures are still being worked out, with the aforementioned recruitments and final implementation of the reorganization, we feel we will have a viable extramural program.

Scientific Highlights of the Neurological Disorders Program

The Neurological Disorders Program supports research in the Developmental Disorders, which includes cerebral palsy, mental retardation, metabolic disorders, genetic disorders, behavioral disorders such as autism, and learning disorders such as dyslexia. Supported research in the Convulsive Disorders is focused on epilepsy and narcolepsy. The Demyelinating Disorders are represented by research in such disease entities as multiple sclerosis, amyotrophic lateral sclerosis, allergic and infectious diseases. Research in the Diseases of the Aging includes work on Parkinsonism, the organic dementias, and memory disorders. Research applicable to the Neuromuscular Disorders includes muscular dystrophy, myasthenia gravis, and the peripheral neuropathies as well as other general muscular disorders. Finally, research on the Infectious Disorders includes work on the encephalopathies, meningitides, and focal infections. There are approximately 600 research grants including the Clinical Research Centers and Program Projects that are administered by the Neurological Disorders Program. 'Direct costs only' research support amounted to about \$42 million during the past fiscal year.

During this past year the President's Commission on the Control of Epilepsy and its Consequences held its first meeting and promises to produce a thorough and analytic report on the country's needs for research and service in this all too common malady. Two notable events in epilepsy therapeutics are the introduction to clinical practice of a new anticonvulsant drug, clonazepam, and valporate sodium, which is expected to come on the market in 1977. To encourage development of additional drugs, the NINCDS has initiated a screening program which enlists the cooperation of medicinal chemists from pharmaceutical companies and universities. Promising compounds are evaluated in a standardized manner for anticonvulsant efficacy and potency in mice. In Parkinson's Disease, a new class of drugs -- called dopamine agonists because they mimic the action of the endogenous neurotransmitter -- is being evaluated. The weakness in myasthenia gravis has now been shown to have an immunological basis resulting from a circulating immunoglobulin which prevents normal muscle stimulation by blocking neurotransmitter access to the muscle receptor. The development of an animal model for the study of this disease is one of the high points of the past two years. As a result of the International Workshop on Diabetic Neuropathy organized last summer by the National Commission on Diabetes and the NINCDS, the Institute is planning to implement the recommendations as outlined in the Commission Report submitted to the Congress. Much of the data from the Multiple Sclerosis Survey in the Shetland and Orkney Islands is currently being evaluated. One aim of the survey was to ascertain whether certain histocompatibility antigens which are inherited in specific patterns are unique markers of diagnostic or screening significance for MS. A Workshop on the Neurological Basis of Autism was held this year with a distinguished international group of invited participants. A Report will be forthcoming during the coming year. Detailed discussion of all ongoing activities of the Neurological Disorders Program will be found in the following sections of this annual report under the headings Grant Activities, Developmental Neurology Branch Report and Epilepsy Branch Report.

CONTRACT NARRATIVE
Neurological Disorders Program
July 1, 1975 - June 30, 1976

CLINICAL NEUROLOGY INFORMATION CENTER AT THE UNIVERSITY OF NEBRASKA
(NIH-NINCDS-72-2300)

Title: The Operation of a Clinical Neurology Information Center

Contractor's Project Director: Walter J. Friedlander, M.D.

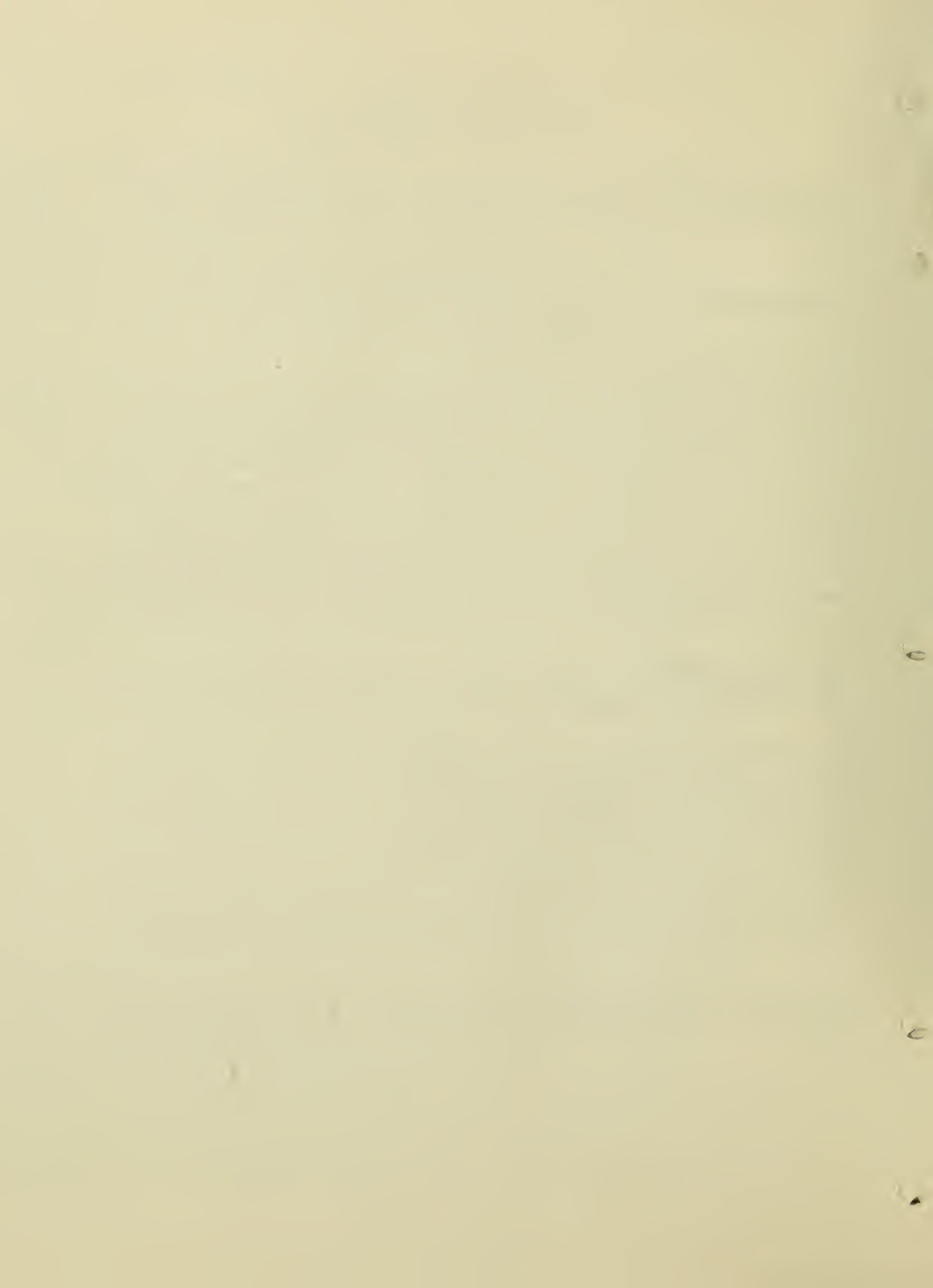
Current Annual Level: \$132,000

Objectives: To operate a specialized Information Center on Clinical Neurology. This Center will be an international focal point for information relating to those diseases of interest to NINCDS, especially information relating to diagnosis, treatment and prevention of diseases of the brain and central nervous system. The Center is producing reviews of various clinical problems of interest to the Government, bringing together the relevant clinical knowledge as it applies to the problem. These reviews may focus on an entire disease problem as a whole, or on any distinct part of a disease entity. The Center will identify sources of information relevant to clinical neurological problems, including indexing services, abstracting services, periodical journals, books, monographs, etc.

Major Accomplishments: In its fourth year of operation, the Center has obtained a number of critical reviews from leading clinical specialists and published them in Advances in Neurology, Volume 13, Raven Press.

The Center's most innovative product and one that has been received enthusiastically by the approximately 1400 scientists who receive it is the Concise Clinical Neurology Review. This publication emphasizes a one sentence (terse) abstract of each paper in a cluster of terse statements on a single topic. A number of these topics are covered in a single issue of the bulletin. The bibliographic citations are referenced by a number and appear together in a second part of the bulletin. Approximately 300 papers are selected for inclusion each month, based on a review of 818 serials. This publication seems to fulfill a need not otherwise met in neurology. It is produced once every two weeks and has a subscription rate of \$15 per year.

Proposed Course of the Contract: This program is under the surveillance of an NINCDS project officer and its performance is under continued review.



ANNUAL REPORT
July 1, 1975 - June 30, 1976
Neurological Disorders Program
National Institute of Neurological and Communicative Disorders and Stroke
National Institutes of Health

GRANT ACTIVITIES

MULTIPLE SCLEROSIS

Multiple sclerosis is one of the more common chronic neurologic diseases in the United States today. More than 100,000 patients are severely crippled by this disorder. The cause of this disease is not known and there is no effective long-term treatment. Since Charcot originally described multiple sclerosis more than 100 years ago, neurologists and neuropathologists have noted an expanded spectrum of clinical and pathologic variation from individual to individual afflicted with multiple sclerosis. In the 1950's, epidemiologic studies indicated both high and low-risk prevalence areas for multiple sclerosis. Further studies on migrant populations suggested that multiple sclerosis was acquired in childhood although clinical disease is not manifested until adult life. While these data were accumulating, several lines of attack were slowly emerging using mainly three model systems; tissue culture, animal models, and whenever possible, human subjects. Multidisciplinary approaches for diagnosis, etiology and treatment of MS currently being investigated include neurochemistry of myelin, myelin proteins and enzymology, neuro-immunology, neurovirology, neurophysiology, clinical neurology and ultra-structural investigations.

The Biochemistry of Myelin: Because myelin appears to be the primary target tissue in this disease, much research has been concerned with its chemistry. Studies of the structure, composition and metabolism of myelin have made it one of the best characterized membrane systems of the body. The lipids of myelin have been particularly well studied but as yet no consistent defect in the lipid composition of myelin from patients with multiple sclerosis has been found.

Myelin Protein: Main components of myelin proteins have been characterized into acid, proteolipid and basic components. Only basic proteins have been studied extensively. One-hundred seventy residue fragments in five species including man and monkey have been identified. Encephalitogenic fragments (45 residues) of this protein were sequenced and the active site (10 residue) was determined and has now been synthesized. Modification of specific residue in myelin basic protein is a major thrust of these endeavors. The objective is to achieve a derivation of myelin basic protein which will not produce EAE, but will be antigenically similar to the native protein in living systems. Use of such modified protein in the prevention and treatment of EAE (and possibly of MS) is obvious. Basic protein constitutes 30% of the total protein of myelin. It has been suggested that it plays a role in the transformation of immature uncondensed myelin-like glial membranes into mature myelin, perhaps by interacting with acidic myelin lipids. Basic protein is a specific marker for myelin and possibly for the myelin-producing cells, the oligodendrocytes. Some of the brain enzymes which are involved in the

synthesis, modification and breakdown of myelin basic protein have been studied and their specificity on model proteins and myelin basic proteins has been determined. The most striking feature of these observations is the marked increase in selective enzyme activity in brains of rats after challenge with basic protein used for induction of Experimental Allergic Encephalomyelitis (EAE); a demyelinating disease. No definite information is available if these enzymes affect the cells or myelin in vivo.

Investigations of animal and human demyelinating diseases have shown that immunological mechanisms are operative in experimental allergic encephalomyelitis (EAE) and may be operative in the human disorders. For example, it has been demonstrated that EAE can be transferred by lymphoid cells sensitized against central nervous system tissue. The encephalitogenic property of the latter residues is the basic protein of myelin. In the human demyelinating diseases the evidence is indirect and less convincing. Nevertheless, consideration is now being given to the possibility of treating multiple sclerosis patients with basic protein in an effort to alter the postulated immune abnormalities. The recent characterization of the basic protein and synthesis of encephalitogenic fragments makes such an approach technically feasible. To provide a more rational basis for such a consideration, it seems important to explore in greater detail the immunological mechanisms underlying the experimental disease and to seek further evidence that such mechanisms operate in patients with multiple sclerosis.

Tissue Culture: Over the past several years a particular tissue culture technique has been applied to the study of experimental allergic encephalomyelitis (EAE) and MS. This technique was first adapted to the newborn kitten and rat cerebellum. Later, it was extended to include almost all of the mammalian neuraxis from the cerebral neocortex to the neuromuscular junction which has proved valuable for examining biological and pathological events since it reproduces in vitro most of the structural, bioelectric and biochemical characteristics of the developing and fully differentiated nervous system. It was observed that remyelination can and does occur in the mammalian CNS when demyelinating antibodies are removed from its environment. Since this remyelination was first observed in tissue culture, several substantiating reports have appeared from electron microscopic examinations of tissue from EAE-exposed animals and MS patients as well as from other kinds of demyelinating trauma.

Having the organotypic, myelinated cultures the hypothesis that the disease MS involved, in fact, an immunological process, an autoallergic encephalomyelitis was examined. The concept of the immunopathology of MS arose at about the beginning of this century from the observations of the similarity between the histological lesions found in patients who had succumbed to post-rabies inoculation encephalomyelitis and those who had MS. The development of EAE as a laboratory model of demyelinative disease produced by immunological means and its extensive application in a number of investigations supplied evidence to support the hypothesis that MS might also be the direct result of an immunopathological process. Demyelination was chosen in culture as the significant end point with which to relate EAE and MS. The experiments involved rabbits inoculated with whole CNS white matter in complete Freund's adjuvant. Blood was obtained prior to, during, and after the onset of overt

manifestations of the neurological disorders. The serum of these animals was added to the culture's usual nutrient medium along with fresh guinea pig serum as a source of complement. This mixture proved capable of reproducing in the myelinated cultures of rat cerebellum the pathological pattern of demyelination with relative sparing of the axis cylinders as seen in MS and most animals with EAE.

After having established the demyelinating response to EAE serum, it was logical that human sera be tested for similar properties. The horizontal study is composed of a select group of patients from whom blood samples are drawn serially from month to month. The samples were examined in culture. In each case, the results were compared with the clinical diagnosis and the clinical states, i.e., exacerbation and remission in the case of this clinical type or chronic progressive form if the patient's illness is characterized by the clinical pattern. To date, the vertical study confirms that about 60% of MS patients' sera demyelinated cultures if the sample had been obtained during a period of clinical activity whereas only about 10% of the sera from MS patients in remission or from normal patient controls had demyelinating abilities. The horizontal study suggests that the presence or absence of demyelinating factors may fall into 2 patterns. In most patients, it is absent or undetectable during periods of remission but appears during exacerbations. On rare occasions was it possible to find the appearance of demyelinating factors prior to the onset of clinical manifestations of an exacerbation. These observations correlate very closely with the demyelinating response with those produced by exposure to EAE serum. As stated above about 10% of serum samples obtained from MS patients in remission, patient controls and normal controls were also found to have demyelinating potency. The sole exception to this finding was the observation that about 60% of the samples from patients with amyotrophic lateral sclerosis (ALS) were also capable of demyelinating cultures. Studies are now underway to characterize the nature of the demyelinating factor in MS sera. The experiments to date support the likelihood that the human (MS) factors are also antibodies. Thus, they are complement dependent as shown by the abolition of demyelinating ability, by heating to 56°C for 1/2 hour, and the re-establishment of potency by adding fresh guinea pig serum as a source of complement. On the other hand, demyelinating potency is permanently decreased by exposing the serum to brain tissue whereas a similar treatment with non-neural tissue (testes) has no such effect. Current studies involve the fractionation of the active MS sera into various immunoglobulin components and the testing of their demyelinating capabilities.

EAE animal model: To date, most of our clinical, immunological and morphological knowledge about MS comes from studying cases with a very long history of the disease; on the other hand, what is known about EAE comes from studying animals during the acute episode 2 to 3 weeks after sensitization to various antigens. Therefore, much of the criticism as to the differences in clinical course and morphology between EAE (by most investigators it is considered as a monophasic disease) and MS (chronic recurrent disease) cannot be properly evaluated because the two demyelinating diseases were studied at very different stages of their history. The objective is to develop chronic recurrent EAE in inbred beagle dogs. Previous studies observed an encephalomyelitis with myelin destruction in monkeys that had received repeated intramuscular

injections of aqueous emulsion and alcohol-ether extracts of normal rabbit brain. This technique produced morphological lesions of various age and size, and gave a very low mortality rate. However, it was very time consuming (3 inoculations a week, up to 85 injections) and difficult to apply to small animals. Since at this time the goal of the experiments was to determine what component of brain tissue caused demyelinating encephalomyelitis, there was a great need for a method which would shorten the time and procedure to induce the disease. This was solved when Freund and McDermott demonstrated a new technique of emulsifying antigens in various adjuvants as aquafor, paraffin oil and heat-killed tubercle bacilli. This technique resulted in the experimental production of encephalomyelitis and the inception of its symptoms with one or two injections of brain emulsion in Freund's adjuvant. In subsequent years, with the use of Freund's adjuvant, it was found that the responsible antigen for the induction of EAE is present in the myelin sheath and was eventually characterized as an encephalitogenic basic protein. Although the introduction of Freund's adjuvant accelerated the identification of the antigen responsible for EAE, it also clouded the relation of EAE to MS by producing an acute demyelinating encephalomyelitis with a mortality rate close to one hundred per cent. EAE, by many investigators, therefore, was considered an acute monophasic disease almost always inducing death in contrast to MS, characterized by exacerbation and remissions and negligible death rate. Recent investigations using beagle dogs are conducted to observe whether EAE should be considered an acute monophasic demyelinating encephalomyelitic disease, or one with recurrent episodes of demyelination similar to that observed in multiple sclerosis.

The research is designed to develop and study chronic and relapsing models of EAE. These models will allow examination of the clinical, immunological and morphological pictures of chronic and relapsing EAE, similar to that observed in MS. Today the favorite hypothesis explaining the relapses in MS is that, irrespective of the primary antigen (virus, encephalitogenic basic protein) the autoimmune type of reaction is responsible for the recurrent episodes of demyelination. According to this concept, potentiality for relapses are continually present in patients with multiple sclerosis. To test this hypothesis, blood and lymph nodes from animals during the various stages of the disease will be examined. Knowing the primary antigen, with which the animals were sensitized, it will be possible to test how long after the last exposure to the antigen it is possible to detect antibodies against it in the blood or lymph nodes. Results from these studies will have great implication on diagnostic tests in multiple sclerosis patients, i.e., lymph node biopsy may be performed during the acute stages of MS in order to detect antibodies against myelin basic protein. There is no doubt that the basic protein present in the myelin sheath is responsible for inducing EAE. However, myelin basic protein induces EAE only when injected with Freund's adjuvant or with other components of the myelin sheath membranes. Injection of basic protein with saline solution only prevents or suppresses EAE. These data indicate clearly that if myelin basic protein is responsible for autoimmunization in MS, other naturally occurring adjuvants must operate to induce demyelinating encephalomyelitis. The rationale behind the outlined experiments is to study factors modifying the encephalitogenic properties of myelin basic protein. It should be stressed that the protective effect of encephalitogenic basic protein injected in saline solution has been studied in short-

term experiments, but the outcome in animals after many weeks or months of injections and long survival time is not known. This information is of great importance since it relates directly to treatment of MS patients, especially since an attempt to treat MS with basic protein has already been made.

Search for virus in MS nervous tissue and other clinical specimens: If multiple sclerosis originates as a virus infection of early childhood that, after an incubation of 15 or 20 years, becomes manifest as a disease of late adolescence or young adulthood, then the successful isolation of an infectious virus from the target tissue, i.e., the central nervous system (CNS) of a patient with fully developed disease or after his demise at autopsy, must depend upon very exceptional circumstances. It may be postulated that in most cases of advanced MS, it is too late to detect infectious virus in its complete form; more probably only the virus genome or a portion of it "lurks" in the cell making its detection extremely difficult. To circumvent this obstacle of time, it would be useful to institute a search for the etiologic agent in MS in materials obtained not from cases at an advanced stage of the disease, but at the time when the earliest manifestation of the disease is detected and when the causative agent (virus) might be more widely disseminated throughout the patient's body and still be infectious.

During the past 2 years, 6 MS brains have been extensively studied by virus isolation techniques. The material was usually obtained early, and the methods used were extensive relying primarily on passage of primary brain cultures, co-cultivation of brain cells with indicator cells, fusing of brain and indicator cells, and immunofluorescence of tissues and cultures for viral antigens. New techniques such as hemadsorption and mixed-hemadsorption and radioimmune assays have been added which could be helpful in detecting incomplete viral products. Although the efforts on 6 MS brains so far have remained unsuccessful, these on-going studies using improved and new methods might result in elucidating, at least in part, viral etiology of MS.

Search for virus in optic neuritis: An attack of idiopathic optic neuritis (ON) has been considered by some to be the first manifestation of MS as approximately 40 percent of MS patients have a history of having had ON at some time, and abnormalities of the cerebrospinal fluid (CSF) encountered in ON are often similar to those observed in MS. It is also possible, through typing for HL-A antigen and MLR antigen, to single out those cases of ON showing HL-A 3 and 7 histocompatibility antigens and LD-7a MLR antigen, as cases that are more likely than others to develop MS in the future. Even though the relationship between ON and MS is still controversial, in the absence of any other well described manifestation of the first attack of MS, it should be useful to pursue the study of the viral etiology of ON.

In preliminary ultrastructural studies with leukocytes from two ON patients, the presence of intranuclear nucleocapsid-like structures in the buffy coat cells was demonstrated. These structures were not found in normal controls but were observed in buffy coat cells of MS patients. Occasionally, these structures were observed in the cytoplasm of damaged cells. The intranuclear structures observed in the ON peripheral leukocytes seemed morphologically similar to the nucleocapsid-like structures of paramyxoviruses recently described in mononuclear cells of the perivascular infiltrates of plaques in MS brain tissue.

The main objective of this study is to establish the viral etiology of ON through (a) isolation of a viral agent from various tissues of ON patients; (b) detection of viral antigen in ON tissue by immunologic means; and (c) observation and identification of virus ultrastructures in peripheral blood leukocytes obtained from ON and MS patients.

Biochemical markers of glial cells involved in myelination and their interaction with viruses: If multiple sclerosis is, in fact, a viral disease, the means by which viruses interact with the human organism to result in demyelination and the clinical picture of multiple sclerosis is not understood. To investigate this, it is essential to have a system in which one can study the interaction of viruses with cells that normally produce and maintain myelin, in the absence of other cell types, immune responses, or systemic influences.

The in vitro approach has been successfully employed in a wide variety of investigations on the function of cells of the nervous system. As discussed in the earlier part of this report, neural cells have been propagated in tissue culture. Organotypic culture of fragments and reagggregates of CNS tissue and dispersed culture of mixed neural and non-neural tissue have been developed as experimental systems. Many functions of neurons, glia and Schwann cells are retained in tissue culture. Neural cells have been infected in vitro with 6/94 and measles viruses. Using this system, the biochemistry of glial cell function and the effect of viral infection is being investigated. Although it is recognized that glial cells are important in neural function, relatively little is known about their function at the biochemical level. In addition, there is confusion about the identity of the "brain cells" utilized for virus isolation, experiments on virus-cell interaction, and in vitro immunological assays. First attempts are to isolate and identify purified populations of glial cells which can be maintained in vitro, and characterize them biochemically in terms of glial cell functions with particular emphasis on the enzymes involved in myelination. The following enzymes implicated in myelination are being investigated:

(1) 2', 3'-cyclic nucleotide phosphohydrolase (CNP): CNP is an enzyme of unknown physiological function which is present at high activity in myelin and only at low activity or absent, in other tissues. Its activity increases in parallel with the accumulation of myelin in developing rodent and human brain in vivo and in vitro and it is only found at low activity in the brains of myelin-deficient mutants of the mouse or in organotypic cultures of rat cerebellum in which myelin synthesis is inhibited with 5-bromodeoxyuridine (BUDR). In the purification of subcellular components of brain, the activity of CNP remains with those fractions identified as myelin or myelin precursor. CNP has been demonstrated in cultures of normal and PML-SV40-transformed human brain cells, rat and mouse glioma cells in vitro, and in some classes of somatic cell hybrids derived from these cells.

(2) UDP galactose: Hydroxy fatty acid ceramide galactosyltransferase (C-Gal-T), and

(3) 3'-Phosphoadenosine-5'-phosphosulfate: cerebroside sulfotransferase (PAPS-CST):

These two enzymes catalyze, respectively, the synthesis of cerebroside from hydroxy fatty acid ceramide and UDP-galactose, and of sulfatide from cerebroside and 3'-phosphoadenosine 5'-phosphosulfate. Since cerebroside and sulfatide are major myelin constituents and are present predominantly, if not exclusively, in myelin, these enzymes have been studied extensively. The activities of C-Gal-T and PAPS-CST rise in parallel with myelinogenesis in normal mice, but are present only in low amounts in myelin synthesis deficient mutants. C-Gal-T activity rises with myelination in organotypic cultures of rat cerebellum, and the enzymes have been postulated to exist predominantly, if not exclusively, in myelin or its precursor oligodendroglial cells. These two enzymes in tissue cultures of brain cells from MS patients and controls have been demonstrated.

(4) Glycerol-3-phosphate dehydrogenase (GPDH): This enzyme has been shown to be present in high activity in brain, with activity rising dramatically during the period of active myelin synthesis. Its activity in the brain can be induced by hydrocortisone and related corticosteroids. Activity is high in white matter, high and inducible in glioma cells but low and uninducible in human or mouse neuroblastoma cells. GPDH is the primary source of glycerol-3-phosphate which is the starting material for phospholipid metabolism and this is believed to be the role of GPDH in glia. The class of glial cells which have high GPDH activity has not been defined, but the temporal correlation with myelination and the important role of this enzyme in the synthesis of phospholipids, which comprise 40-50% of total myelin lipid, suggest an important role for GPDH in the myelin-bearing cell.

Effect of in vitro infection on biochemical markers of glial cell function: If multiple sclerosis is indeed a viral disease, the means by which virus infection leads to the clinical and pathological picture of multiple sclerosis is unknown. Evidence is accumulating that one aspect of the virus-induced pathogenesis of MS involves the immune system but nothing is known about the direct effect of viruses on the (presumed) ultimate target of the disease mechanism, the myelin producing cell. In fact, nothing is known whether the disease process ever involves direct interaction between virus and glia or nerve, but since this is a logical possibility it requires testing in a system where interaction with other cell types can be prevented.

The availability of human and animal glial cells which express glial function in vitro, obtained and characterized, makes it possible to study the earliest stages in the infection of glial cells with viruses, and to study these effects in the absence of contributions of the immune system, other cell types or similar complicating factors. In this way, one expects to obtain valuable information on the course of viral infection in those cells and on the resulting effects on glial cell function, particularly as it relates to myelin synthesis, maintenance, and turnover. Pilot experiments utilizing one paramyxovirus (6/94) and one bovine and several human brain cell lines to test the feasibility of this approach have been performed. This work has indicated that UV-inactivated or detergent-killed parainfluenza virus injected intracerebrally (but not peripherally) into mice can produce a neurological disease similar to that produced by live virus. While this is probably an immune-mediated effect, direct effects of viral membranes on cell surfaces are also possible, and may in fact precipitate the immune reaction. Virus-infection experiments in vitro using UV-or chemically-killed virus will be conducted.

Purified populations of glial cells which have been characterized for glial biochemical functions will be infected with virus and followed serially for the effect of the virus infection on glial functions. Initially, these experiments will be done with 6/94 and measles viruses, and the experiments will be repeated with any new viral isolates obtained from MS and ON material.

Hydrolytic enzymes relevant to MS: Hydrolytic enzymes of lipids, proteins and lipoproteins have been long suspected of playing a prominent role in MS. Earlier work was conducted on acid proteinases and other lysosomal hydrolases in demyelination, in EAE and in MS plaques. This work was undertaken because a special role has been postulated for acid proteinase in MS pathogenesis on the basis of the elevated activities of this enzyme, in and around MS plaques and in some cases also in normal-appearing MS white matter. It was speculated that acid proteinase may initiate myelin breakdown by digesting the basic protein, and/or that such partial hydrolysis may release immunogenic fragments which may propagate the process. It thus became necessary to answer the question of whether the higher enzyme levels found are a primary factor in myelin breakdown, or whether they merely reflect the activities of invading lymphocytes and/or phagocytes, or a general activation of lysosomes commonly found where there is tissue destruction.

Lipase-esterase: A lipase-esterase hydrolyzing 4-methylumbelliferyl oleate at pH 5 is decreased in MS plaques by about 65%, whereas in the lesions of EAE it is elevated. This raised the possibility that this enzyme is localized either in the myelin or in oligodendroglia cell bodies or in both. Circumstantial evidence suggested the presence of this enzyme to be localized primarily in oligodendroglia. Recent experiments carried out on oligodendrocyte preparations separated in bulk quantities indicated that this may indeed be a marker enzyme for oligodendrocytes. For this reason it appears desirable to study this enzyme further to define its localization and study those properties which appear relevant to MS.

Cathepsin A: This enzyme is usually measured with CBZ-glutamyl tyrosine as substrate. The released tyrosine is measured fluorometrically and is related to enzyme activity. It has been reported that cathepsin A is increased in MS to a degree much greater than other hydrolases, the same has been found in EAE. Preliminary evidence indicates that this enzyme is highly activated in or near the MS plaque, but is not very active in brain tissues. Using modified assay technique the activities of cathepsin A will be determined systematically in MS, brains, serum and CSF. A change in lipase or protease activity has long been postulated as a possible initiating event in myelin breakdown. Previous work has convinced investigators that acid proteinase (cathepsin D) probably does not play a major role, while Cathepsin A, because of its possibly dramatic increase, still needs to be checked with reliable methods. Lipolytic enzymes, on the other hand, are of interest for many reasons: (a) they may liberate lyso-compounds which lyse membranes; (b) they liberate other lysosomal enzymes by damaging the lysosomal membrane; (c) they are activated by neurohumors; or (d) (as in the case of lipoprotein lipase) released from tissues in response to substances such as heparin. Moreover, an enzyme which may be localized in oligodendroglial cell bodies is of interest to MS researchers, as it would be an invaluable tool in attempts to isolate these cells in order to study their activities.

Search for measles virus in multiple sclerosis: On the basis of pathological, epidemiological, and immunological studies, an infectious etiology for multiple sclerosis has long been an attractive concept. The agent which is mentioned most often in this context is measles virus, and although the evidence is still circumstantial, when all facets are taken together, they are compelling enough to be taken very seriously. Therefore, it is quite possible that at least some cases of multiple sclerosis are caused by measles virus, and various pathogenetic mechanisms involving virologic and immunologic phenomena acting alone or in combination have been proposed as explanations. Of the evidence which attempts to link measles virus and multiple sclerosis, immunologic correlations derived from several studies, and the now well established fact that this virus is responsible for subacute sclerosing panencephalitis, are the most compelling. It is also important to note at this juncture that morphologic studies involving standard histological, immunofluorescent, or ultrastructural methods have largely been inconclusive, and the virus has never been isolated from affected brains. In addition, there have been no reports of searches for measles genetic information in these tissues. Thus, direct evidence for the presence of either measles virus or its genetic information in brain material derived from individuals afflicted with multiple sclerosis is not at hand. From all of this, it can be concluded that there is a considerable amount of circumstantial evidence which suggests that measles virus is etiologically related to at least some cases of multiple sclerosis, but there is no direct evidence for continued presence of measles virus genetic information in sclerotic brains, even though statistically higher levels of measles virus antibody in the serum and CSF of MS were detected in comparison to control population.

The method involves application of single phase (liquid) nucleic acid hybridization techniques with specific aims to employ this RNA as a probe in RNA-RNA hybridization experiments to determine whether the complementary strand (the measles genome or a portion thereof) is present in nucleic acid preparations derived from appropriate areas of sclerotic brains.

Viruses: Scientists from several disciplines increasingly agree that a virus or viruses associated with aberrant host immune responses are likely to be involved in the pathogenesis of multiple sclerosis (MS) and probably amyotrophic lateral sclerosis (ALS). This evidence results from immunologic, virologic, pathologic and experimental findings. The theory that tissue injury in these diseases stems from viral infection associated with an immune response to that infection is based on the following immunologic findings in patients with MS: (1) titers of antibodies against measles virus in the sera and cerebral spinal fluids; (2) clones of antibodies against various structural components of measles virion; (3) disorders of MIF migration and lymphoid tests when lymphocytes from MS patients are incubated with measles virus or other paramyxoviral antigens; and (4) immunogenetic correlation of HLA-3, HLA-7, and LD-7A markers with MS. Virologic evidence comes from: (1) serological surveys mentioned above of association of measles virus infections with MS; (2) isolation of parainfluenza-1-like virus from brain cultures of two MS patients; (3) finding virus or virus-like agents in chronic and degenerative disorders of man and animals, i.e., Kuru, Jacob-Creutzfeldt, subacute sclerosing panencephalitis (SSPE), progressive multifocal leucoencephalopathy, visna, scrapie, Aleutian disease of mink and murine

infections with lymphocytic choriomeningitis virus (LCM), lactic dehydrogenase virus, oncornavirus (WM1504); and (4) canine distemper virus, a virus related to human measles virus, may occasionally cause demyelination of canine brain tissue. Pathologic evidence also supports the infectious-allergic theory by way of the classic observations that inflammatory cells surround blood vessels in areas of recent or active plaques of demyelination.

Mechanisms of viral injury: If these diseases or other similar disorders are caused by a virus or viruses, then injury could result by at least two major mechanisms, which need not occur independently. First, immune responses directly against virus or viral coated or unmasked tissue antigens could result in tissue injury. This possibility has been actively explored over the past several years with many of the mechanisms delineated. Second, the virus itself could slowly alter the biochemical functions of specialized cells in the nervous system without destroying the cell. For example, could MS be caused, in part, by a persistent infection of oligodendroglia cells with resultant dysfunction of enzymes or products needed to form or maintain myelin? Host, viral or other environmental factors may alter the replication of infectious virus with cycling of infectious and defective virus, latency and viral activation, explaining both chronic progressive degenerative disease or disorders with remissions and exacerbations. Further, virus itself may alter membrane structures, functions and affect cells of the immune system by as yet unexplained ways causing on one hand aberrant hyperimmune responses and on the other, immunosuppression. Hence a clear understanding of the immunologic and virologic aspects of chronic disease, careful study in model systems and extension of these findings to human disorders may well provide insight into the mechanism of injury in MS, ALS and related disorders.

As an illustration, normal neuroblastoma cells (N115 and S20), in vitro synthesize norepinephrine and acetylcholine from labeled tyrosine and acetyl-coenzyme A and are able to elicit a spike discharge. These cells were infected with lymphocytic choriomeningitis (LCMO virus, a relatively non-cytopathic virus that is associated with persistent infection in vitro and in vivo and resultant chronic degenerative disease in vivo). Preliminary studies indicate that persistent infection of neuroblastoma cells are associated with significant suppression in the manufacture and/or breakdown of acetylcholine. Despite a significant suppression of these cell functions (making of neurotransmitter enzymes), the cells infected with LCM virus have the same growth rate and cloning efficiency as non-infected cells. It is now planned to study whether these alterations of choline acetylase (CAT) and acetylcholine esterase (AChE) on persistently infected cells are unique for these enzymes or are seen with other cellular enzymes as glucose-6-phosphate dehydrogenase and in cellular metabolism. Further it is important to know whether alterations in neurotransmitters occur in each cell in the population or just to a few selected cells. The N115 and S20 lines are chosen for study on the basis of preliminary experiments which showed significant reduction in AChE and lowering of CAT in infected N115 and significant reduction in CAT in infected S20 lines when compared to non-infected controls.

Defective interfering (DI) virus: One of the important aspects of virus host relationship is to observe whether virus infecting host cell retains its complete integrity or does it undergo transformation. Defective viruses are

those viruses which have reduced (if any) ability to singularly initiate infection in a cell and produce progeny capable of doing the same. Defective virions may lack or have temperature sensitive defects in structural elements in the capsid or envelope, or they may have alterations in their genetic information. For instance, a persistently infected culture producing defective virus lacking virion "spikes" may be less susceptible to immunological attack. However, it seems obvious that the most important class of defective viruses involved in chronic infections may be the defective-interfering (DI) kind, or those which are capable of interfering with the replication of the standard viruses from which they are derived. Most DI viruses that have been studied contain standard viral structural protein and a genome deletion. The shorter nucleic acid in such viruses is thought to compete with the longer S genome at the levels of transcription, replication, encapsidation, or generalized use of intracellular proteins. This competition results in enrichment for DI virus in cultures infected with both DI and S virions. Cells infected with DI viruses alone are incapable of liberating infectious progeny. Of great importance in this kind of investigation is not only to observe whether these DI particles can produce functional or morphological changes of neuronal cells, but to change invading viruses into their defective components so as to render them ineffective in propagating the human neurological diseases. Several different viruses (LCM, measles, WMU-1504, Sendai and MHV) are being used in a variety of cell lines and strains of animals. Attempts to activate or recover virus from "latently" infected cells involve cell fusion, blind passages, various drugs (e.g., Actinomycin D, mitomycin C, cyclohexamide, BUDR) and mitogens (lipopolysaccharides, plant lectins) which alter the normal cellular metabolism.

Viral induced mechanisms in demyelination: Demyelination refers to the selective destruction of myelin sheaths with relative preservation of axons. Demyelination can be induced by direct injury to the myelin lamellae or indirectly by the destruction of the myelin supporting cells, i.e., oligodendrocytes in the central nervous system (CNS) and Schwann cells in the peripheral nervous system (PNS). By electron microscopy it is possible to observe distinct early changes characteristic of certain disease states affecting either the myelin supporting cells, the myelin lamellae or both: e.g., Schwann cell changes due to alterations of plasma membranes, focal cytoplasmic degradation are recognized as the earliest visible pathology in diphtheria, tellurium and lead neuropathy respectively. On the other hand in allergic neuritis (EAN) and idiopathic polyneuritis or the Guillain-Barre syndrome in man, Schwann cells do not degenerate but myelin lamellae is recognized as a specific demyelinating pattern whereas the invasion of myelin sheaths and the stripping of myelin lamellae by macrophages are nonspecific, though more spectacular changes that always occur in more advanced states of demyelination regardless of whether sheaths or supporting cells are primarily affected.

In virus induced demyelinating diseases, the following mechanisms may be responsible for demyelination: (1) the myelin sustaining oligodendrocytes are destroyed by the direct cytotoxic effect of the virus; (2) destruction of infected oligodendrocytes and/or myelin sheaths are damaged by cytotoxic factors released during immune reactions; (4) myelin lamellae are destroyed by an autoimmune reaction in a similar fashion as observed in allergic encephalomyelitis in which the host becomes sensitized to myelin specific proteins. This latter hypothesis implies that a virus infection either releases specific

sensitizing factors, incorporates antigens into myelin proteins and/or plasma membrane of oligodendrocytes or uncovers hidden antigens which then become the target of an allergic reaction.

Morphologic evidence suggests that chronic relapsing demyelination in multiple sclerosis begins with a destruction of oligodendrocytes. It is not known whether these cells are destroyed by virus infection, autoimmune mechanisms or both. Acute MHV infection in mice produces an encephalomyelitis in which oligodendrocytes are selectively destroyed. There is suggestive evidence that persistent infection occurs and that virus reactivation can be accomplished following thymectomy. Studies are aimed at determining the susceptibility of mice to acute and persistent MHV infection by selecting strains that differ in their histocompatibility and immune responsiveness. The localization of virus and immune complexes as well as the patterns of demyelination in persistently infected mice subjected to modifications of their immune responsiveness are to be investigated. The in vitro studies are expected to demonstrate morphologic changes that occur in infected cells in the presence of antibody which might provide clues to the understanding of virus persistence. Evidence of altered cell function due to persistent virus-infection exists. The in vitro studies on various cells following different virus infections are aimed at demonstrating morphologic correlates to the observed functional alterations. The investigations on the fate of virus-antivirus antibody complexes on the cell surface will contribute to the understanding of virus persistence.

Other viruses implicated in MS etiology are discussed in a separate section of this report dealing with "Viruses and Neurological Disorders."

AMYOTROPHIC LATERAL SCLEROSIS

Amyotrophic lateral sclerosis (ALS) is a progressive, grave disease of unknown etiology, patients generally dying within three years of onset. Its incidence is almost equal to that of multiple sclerosis. Most of the cases are sporadic, although there are rare families in which a similar disease is inherited as a dominant condition. It is a primary disease of the motor neurons and demyelination is usually a secondary feature.

Clinical types of ALS: ALS, a motor neuron disease, is due to degeneration of pyramidal cells in the motor cortex, motor neurons in the brain stem or anterior horn cells in the spinal cord associated with cortico-spinal tract degeneration. This disorder has been sub-classified clinically as progressive muscular atrophy, progressive bulbar palsy and amyotrophic lateral sclerosis. These three forms of ALS are referred to as the "sporadic" or "classical" form of ALS. (1) In the United States this form is characterized by an annual incidence rate of 1 per 100,000 with median age of onset of 57 years and the estimated clinical course of 4 years duration. The etiology of the disorder at this time is speculative. Factors such as triortho-cresyl-phosphate, organic compounds of mercury, pancreatic dysfunction and slow viruses have been proposed. Attempts to isolate a transmissible agent have not yet been successful. (2) A "familial" form of ALS has also been described affecting a total of approximately 60 families. The clinical course is similar to the classical type of ALS but there is in addition to the pathological findings noted above, demyelination in the posterior columns and spino-cerebellar tracts and degeneration of Clarke's Column. (3) A form of ALS (Western Pacific Type) is seen in Guam, New Guinea, and the Kii peninsula of Japan with differences from both the "classical" and "familial" types of ALS. In these areas (particularly Guam), a prevalence rate of 100 per 100,000 with an average annual incidence death rate of 32.3 per 100,000 is found. Ten percent of adult Chamorro deaths are due to this disease. In the USA, the average mortality rate from ALS is 1 per 100,000 while the Guam death rate due to ALS is 108 per 100,000 for males and 42 per 100,000 for females. This is 50 to 100 times the rate observed elsewhere in the world. In addition to these epidemiological differences, the patients are younger than those seen in the "classical" form of ALS and survive somewhat longer. The tissue changes are those of the "classical" type of ALS but in addition there is diffuse neurofibrillary degeneration of CNS neurons. Another neuronal change has been associated with the types of ALS seen in Guam and is related to the Parkinson dementia complex which also occurs at a high rate on the island. The additional change is the presence of neurofibrillary degeneration; a disorder of the neuronal fibrous proteins. This change is seen in many diseases of the aging, and can be produced experimentally in vitamin E deprivation, administration of vincristine, aluminum, colchicine as well as a biological response of the cell body to axonal section.

Although there is a similarity of these three types of ALS disorders, the underlying pathogenetic mechanism may be dissimilar. No single concept of the cause or pathogenesis of ALS has been widely accepted and it is reasonable to assume that ALS may be more than a single disease.

Although amyotrophic lateral sclerosis has been known and studied since the nineteenth century, its etiology and pathogenesis remain obscure. Various

etiologies have been proposed: (1) Some observers have suggested that ALS is a degenerative disease or abiotrophy. (2) There are neurotoxins which mimic the pathology of ALS in experimental animals, and it has been suggested that there may be exposure to some ALS patients to a neurotoxic substance, particularly in those cases of ALS found in high incidence among the Chamorros of the Mariana Islands. (3) Because abnormalities of pancreatic function have been reported in ALS, a deficiency of some essential nutritional element(s), such as vitamin E, has been postulated. (4) Abnormalities of collagen have been found in the dermis of ALS patients which could indicate an abnormality of nerve growth factor (NGF) in these individuals, since fibroblasts do produce NGF in tissue cultures. (5) An association of spinal anterior horn cell pathology with carcinoma has been reported, raising the possibility that ALS may occur as a remote effect of carcinoma, on a toxic, immunological or viral basis.

ALS and WM1504 (C Type) virus: In 1973 Gardner and his associates described a neurotropic C type virus. This virus caused a high incidence of spontaneous lower limb paralysis in a population of wild mice and isolation of that virus (WM1504) induced a similar disorder when infected into newborn laboratory mice. The virus was identified as a type C oncornavirus and appeared to have a direct neurotropic effect upon anterior horn neurons located predominantly in the lower spinal cord. These observations then represented a naturally occurring disease of anterior horn cells caused by a virus which could be passaged and studied in the laboratory. Studying these mice clinically and by light and electron microscopy it was determined that not only were the anterior horn cells involved but also neurons in the dentate, hypothalamic and occasionally cerebral cortex. Electron microscopy clearly showed the presence of C type virus in various replicative stages in these neuronal cells.

In order to critically observe immunopathology of WM1504 virus, 12 different strains of inbred mice will be inoculated at birth with standard inoculum of purified WM1504 virus. Animals will be followed clinically for occurrence of hind limb paralysis and other aberrant manifestations. Tissue will be studied with light microscopy, EM and immunofluorescence to look for host immunoglobulin, complement, fibrinogen and evidence of viral antigen deposits. This approach, hopefully, will determine whether immune complex deposits occur in mice infected with WM1504 virus and what the specificities of the deposited immunoglobulin and antigens are.

Preliminary studies demonstrated a strain susceptible to disease by WM1504 infection. Susceptible C57/Br mice developed severe anterior horn cell disease and showed marked elevations in viral content in the spinal cord and elevations of 9S antigen in spleen. In distinction, the resistant C57/B was relatively disease free and showed little virus as p30 antigen. This relationship to mouse strain will be further studied in various mouse strains selected on the basis of their histocompatibility type. These studies will also employ congenetic mouse strains and will attempt to correlate disease susceptibility with histocompatibility markers. Specifically, the studies will look for an association with the H-2 as IR gene. If such an association is found, this could then be related to either the effect of the immune response to the infection or to viral receptors. The importance of the immune response will also be studied using immunized mice and transfer systems and parabiosis with immune mice. In addition, immunopathological disease will be studied with

respect to immune complex functions. The role of thymic function to disease will be studied using Nu/Nu mice. This will involve studies of both anterior horn cell disease and tumor production to examine the importance of the cell for virus production. Assays of gs antigen necessary for these studies will also be applicable to human studies in patients with ALS and will employ a quantitative radioimmune assay. Further hybridization studies of viral nucleic acid with infected and non-infected mice will be undertaken.

These studies should provide valuable information on host susceptibility in this model employing an oncornavirus infection. This is of particular importance in view of the similarities of this model to ALS in humans. Consequently, the studies of genetic susceptibility and the role of the immune response is of considerable importance.

ALS and poliomyelitis: A recently reported study has shown that a neurogenic hind limb paralysis can be produced in mice infected with a nontransforming oncogenic type C virus strain. The same virus is capable of inducing lymphoma when inoculated into mice. The neurological disease appears to result from viral infection of anterior horn cells of the lower spinal cord, causing a vacuolar neuronal degeneration and spongiosis, with little inflammation and no demyelination. The type C virus particles appear to replicate within the rough endoplasmic reticulum of the anterior horn motor neurons. This neurological disease of mice may prove relevant to ALS, and is particularly intriguing in view of the reported association of ALS with neoplasia, and the demonstrated production of nerve growth factor by type C virus-containing mouse L cells. In view of the evidence, as in SSPE, of apparent reactivation of a latent viral agent which seemed to have been combated effectively years before, a reported association of ALS with poliomyelitis is intriguing. A number of cases have been reported, and several have been seen in clinical experience, in which ALS or a similar but more benign form of motor neuron disease has appeared many years subsequent to recovery from apparently typical poliomyelitis; and a retrospective study of 196 ALS patients found a history of previous poliomyelitis in five patients. It has been reported that ALS patients on Guam had the same serum antibody levels to poliovirus as did control subjects; and a recent study of 34 sera from ALS patients and 42 control sera showed no significant differences in complement fixation (CF) antibodies to polioviruses, types 1, 2 and 3. Presently neutralizing (Nt) antibodies are being studied in serum and CSF and, particularly, cell-mediated immunity to poliovirus in a group of ALS patients and in controls, and also to correlate these findings with genetic data derived from histocompatibility typing.

MEV and DA viruses: Another approach to studying a possible association of poliovirus infection with chronic motor neuron disease, stems from working with various strains to Theiler's Mouse Encephalomyelitis Virus (MEV) in an effort to establish an experimental model for ALS in the mouse. The similarities of mouse to human poliomyelitis are striking: Both diseases are caused by picornaviruses; both are usually inapparent enteric infections; but, in both, flaccid paralysis with necrosis of motor neurons in spinal cord and brain may occur. It is to be noted that latent infection with MEV is not uncommon, although spontaneous overt CNS disease occur rarely (1 to 10 per 10,000 mice); and that MEV is occasionally isolated following IC inoculation into mice of tissue homogenates or viral agents of diverse origins, with many of these animals

manifesting frank encephalomyelitis. Moreover, another mouse enteric virus, designated DA, has been shown to produce a chronic, spastic CNS disease in older mice (age 6-8 weeks) with destruction of myelin in lateral, ventral and posterior columns of spinal cord and degeneration of ventral roots, but minimal gray matter pathology. Given the similarities between the human and animal diseases, it has seemed reasonable to postulate that reactivation of latent or inapparent poliovirus infection can occur in man. Such reactivation, which need not be accompanied by an inflammatory response, could cause subacute and chronic disease of motor neurons, i.e. ALS. It is proposed to continue these studies of MEV and DA virus infection, attempting to establish a reproducible animal model for ALS.

Presently, studies are being conducted on poliomyelitis caused in mice by the MEV (GD-7 strain) and DA viruses, in an effort to establish an experimental model for ALS. Particular emphasis will be given to the DA virus, which has produced the most promising results to date. The distribution of viral antigen will be studied in chronically diseased animals, using fluorescent antibody techniques. Spinal cord and chronically diseased animals, using fluorescent antibody techniques. Spinal cord and brain sections will be studied under the electron microscope. Attempts will be made to recover virus from chronically diseased animals by applying techniques of activation and rescue of latent virus to tissue cultures of CNS. Attempts will be made to alter the course of disease by employing anti-viral sera, anti-viral chemotherapeutic agents (such as guanidine, isoprinosine and 5-fluorouracil), and immunosuppressive agents. Organ cultures of neonatal mouse spinal cord will be used to study the effects of the MEV and DA viruses on organized nervous tissue in vitro. Cultures will be infected with virus and studied morphologically, by fluorescent antibody methods and under the electron microscope. The protective or toxic effects of anti-sera to the virus will also be studied.

Herpes simplex and mumps viruses: Two other viruses, herpes simplex and mumps, have been linked to ALS. The possibility of a relationship between HSV and ALS is especially interesting for several reasons: 1. It has been established that this virus can persist in the brain in a latent state after encephalitis in rabbits and can be reactivated by administration of epinephrine or immunosuppressive agents weeks to months later. 2. Intradermal administration of HSV to mice in a hind limb footpad, can cause a paraparesis with virus present in a latent state in spinal ganglia and recoverable only after these ganglia are grown in organ culture in vitro. 3. HSV is present in trigeminal ganglia of apparently normal individuals as well as of those afflicted with trigeminal neuralgia, and tissue culture methods are required to detect and recover the virus in this situation as well. 4. Recent results indicate that recurrent HSV infection can cause recrudescence of experimental auto-allergic encephalomyelitis (EAE), an apparent interaction of viral and immunological mechanisms to produce CNS disease.

Mumps virus has been implicated in ALS by the observation that a history of mumps in adult life is frequently recounted by ALS patients. A recent study has found no increase of mumps hemagglutination-inhibition (HI) antibodies in ALS sera but the possibility of an aberrant mumps infection prior to ALS has not otherwise been explored further. The question is of some interest because of the known propensity of mumps virus to attack the salivary glands and

because of the relationship of the salivary gland to nerve growth factor (NGF), which will be discussed later.

Further relationships of HSV and of mumps virus to ALS in ALS patients will be explored in experimental animals and in tissue culture systems. Since HSV-2 has been demonstrated to be more neurotropic in laboratory animals than is HSV-1, HSV-2 may prove the more useful strain for these studies which will include tests for antibodies to these viruses, postulating that these will prove more specific than CF or HI tests.

Additional evidence of viral etiology in ALS: Additional, although less direct, arguments can be marshalled in support of a viral and/or immunological etiology of ALS: 1. Eosinophilic inclusion bodies are seen in the cytoplasm of affected anterior horn cells of some ALS cases and they are similar in appearance under the light microscope to the Lyssa bodies seen in rabies. 2. Anterior horn cell loss histopathologically similar to that occurring in ALS also occurs in some cases of Creutzfeldt-Jakob disease, which has been demonstrated to have an infectious basis. 3. The findings of clusters of ALS cases on Guam and in some families imply an extrinsic and possibly infectious etiology, and/or a genetic predisposition. 4. A group of Russian researchers has reported transmitting an ALS-like disease from humans to rhesus monkeys, by inoculating spinal cord extracts; these studies have not been confirmed by workers in this country. 5. Deposits of immunoglobulin (IgG) and complement (C₃) have been found in renal glomeruli of some ALS patients.

Rationale for search of viral etiology in ALS and its relation to MS: In recent years, infectious agents have been found in a number of CNS diseases that had an unknown etiology. Examples include Kuru, Creutzfeldt-Jakob disease, subacute sclerosing panencephalitis and progressive multifocal leukoencephalopathy. The finding of infectious agents confirmed the contention held by many workers that these diseases fit the pattern of slow infections as described by Sigurdsson. Some of the characteristics of ALS are consistent with a slow infection. For example: 1. It has a protracted and irreversible course ending in death, and 2. It primarily affects a single organ. Furthermore, some of its epidemiological features also suggest viral etiology. To date, however, there is no direct evidence for an infectious agent. The essential approach of these earlier studies was to look for obvious effects of virus, i.e., disease in animals and overt CPE in tissue cultures. Although the search for an infectious agent in ALS has thus far failed, this should not deter fresh efforts with new approaches. In the fields of slow infections and cancer research, there have been a number of recent developments which provide more sensitive means of detecting and "unmasking" an infectious agent. These new techniques include: (1) cocultivation of cells (2) cell fusion (3) using DNA inhibitors to induce the synthesis of latent virus, and (4) neonatal animals as a particularly susceptible host. These and other approaches have proven to be extremely successful with MS. There are a number of similarities between MS and ALS. These include: 1. a strong immunological component in each disease as evidenced by (a) migration inhibitory factor (MIF) positive reactions and (b) possible immune complexes in the kidney. 2. demyelinating factors in serum and CSF. Although there are these similarities, there are a great many profound differences in the histopathological appearance and in the clinical findings. However, the point to be stressed is that these indirect

approaches which have been so fruitful with MS could prove fruitful in detecting infectious agents associated with ALS.

Isolation of viral agents from human tissue: The possibility that viral agents may be carried by lymphocytes has been shown in studies of SSPE, scrapie and related diseases, and suggested by immunological studies of MS. Peripheral blood lymphocytes from ALS patients will be grown in tissue culture, utilizing established methodology. Culture media will be assayed in indicator tissue culture cells for presence of virus, and attempts will be made to activate a virus by cocultivation or fusion of lymphocytes with potentially susceptible cell lines. Lymphocytes will be examined by fluorescent antibody techniques and under the electron microscope, to detect presence of virus. Agents such as phytohemagglutinin, pokeweed mitogen and Concanavalin-A will be added to cultures in attempts to activate latent viral agents. If studies on peripheral lymphocytes prove promising, similar methods may be applied to lymphoid tissue obtained from ALS patients at autopsy or at times of surgery performed for conditions unrelated to ALS, explanted and grown *in vitro* in tissue culture. Attempts will be made to identify and isolate viral agents from human CNS tissue obtained at autopsy from ALS patients, as such material becomes available. Explants will be grown in tissue culture, cytopathic effects and viral antigen looked for, techniques for rescue of latent agents applied and experimental animals will be inoculated.

A particular interest is shown in exploring the salivary gland as a possible site of chronic viral infection in ALS. This interest is based on speculations concerning a role of NGF in motor neuron disease, on the probable site of NGF synthesis in the mouse salivary gland, and on the increased reporting of adult onset mumps on ALS patients. Attempts will be made to obtain salivary gland tissue from ALS patients at autopsy, to examine it morphologically and to attempt identification of viral antigens and isolation of viral agents by methods similar to those applied to CNS tissue. Saliva will be collected from ALS patients and assayed for virus and for antibodies to virus.

Histocompatibility types and CNS disease: It is believed that recent observations of alterations in the distribution of histocompatibility antigens in multiple sclerosis may prove relevant to ALS. Histocompatibility antigens are genetically controlled cell surface structures which differ between individuals and are defined by the fact that grafts exchanged between individuals differing in these antigens are rejected. Besides their obvious role as determinants of individuality several lines of evidence point to additional biological significance for these cell surface structures. Thus, they have been implicated in the control of the immune response to synthetic antigens and in susceptibility to experimental autoallergic diseases, such as thyroiditis and EAE, and they are related to the susceptibility of experimental animals to oncogenic viruses. In man, the major histocompatibility system is called HL-A. Certain HL-A antigens have been linked to MS, and to other diseases of presumed auto-allergic and/or viral etiology (e.g., systemic lupus erythematosus, Hodgkin's disease, psoriasis, and adult coeliac disease). In all these diseases, and in ALS also, a slight but definite familial predisposition to disease has been described. Studies have shown a significantly increased incidence of the HL-A antigens, HL-A3 and HL-A7, (39% vs. 21.0%) in MS. Increased titers of measles antibodies found by many workers in MS sera seem to correlate with the increased incidence

of HL-A3, rather than with MS per se; thus, measles antibody titers are increased in HL-A3-bearing controls, as well. Inasmuch as histocompatibility antigens are cell surface components the suggestion has been advanced that they may provide (or fail to provide) receptor sites for certain viruses. Also, certain viral antigens may mimic histocompatibility antigens and thus not be recognized as foreign by hosts of appropriate genotype. Thirdly, the effectiveness of immune response to viral agents may be determined by immune response genes which are linked to histocompatibility determinants. Thus, it is possible that susceptibility to poliomyelitis, for example, may be determined in part by the HL-A makeup of the individual. It follows that the study of HL-A types in ALS has immediate relevance for investigation of possible viral or immunologic etiology and may clarify the status of familial cases. The studies of histocompatibility antigens in ALS to correlate those data with determinants of humoral and cell-mediated immunity to poliovirus, HSV, mumps or such other candidate viruses may be suggested by these investigations.

Neurotoxic factors in ALS serum: There is evidence that circulating factors in the serum of patients with ALS can cause demyelination of myelinated neonatal rat cerebellar explants in tissue culture, and destruction of anterior horn motor neurons when added to fetal mouse spinal cord explant cultures. Whether these effects depend on the presence of complement is being explored currently. Moreover, it has been reported that complement-fixing antibodies to brain are present in serum in 50% of ALS patients. It is proposed to determine the nature of the toxic substance present in ALS serum, which might be an antibody to a surface component of motor neurons. Studies of the factor in ALS serum which causes demyelination and motor neuron destruction in CNS organ cultures will be investigated. Neonatal mouse organ culture assay system will be employed for detailed morphological and electron microscopic study of the motor neuron destruction. Immunofluorescent methods will be applied in attempts to identify the site of binding of the serum factor and to ascertain whether this is an immunoglobulin. "Purified" gamma globulin will be prepared from ALS sera and tested in the organ culture system. Attempts will be made to purify and characterize chemically the "toxic agent." In order to determine whether the neurotoxic effect is specific for anterior horn neurons, ALS sera will be tested in organ cultures of mouse sympathetic and sensory ganglia.

Nerve growth factor (NGF): Nerve growth factor (NGF) is a basic protein of molecular weight 13,500. It is found in considerable quantities in the male mouse submaxillary gland, and may be synthesized there. It has the property of inducing rapid and extensive neurite outgrowth when applied in nanogram quantities in vitro to explanted embryonic sensory explants of sympathetic ganglia. Sensory ganglia are most sensitive to NGF during a restricted part of the embryonic period (chick), while sympathetic ganglia remain sensitive throughout life. NGF also exhibits profound effects in vivo. For example, newborn mice injected daily with NGF exhibit hypertrophy of their sympathetic ganglia, and this enlargement appears to be due to an increase in both neuron size and number. While NGF displays a variety of metabolic effects, it is not yet known whether any of them reflect the primary action of the growth factor. For example, NGF enhances glucose utilization, lipid synthesis, protein synthesis, and RNA synthesis in susceptible nervous tissue. Several lines of evidence suggest that the primary effect of NGF is exerted upon the protein synthetic machinery. The biological significance of NGF remains unclear.

Injection of an antiserum to NGF into newborn mice produces a massive destruction of the sympathetic nervous system, a process termed "immunosympathectomy." The availability of NGF antisera has also made possible the development of sensitive radioimmune assays for NGF, which have confirmed its presence in highest concentration in the submaxillary gland of adult male mice. Significant quantities of NGF are also present in the sympathetic ganglia, kidneys, adrenals, vas deferens and sera of mice. Lower, but detectable levels are present in liver, spleen, muscle, brain and heart. It is not clear where NGF is synthesized *in vivo*. Although highest levels are found in the submaxillary gland, and there is evidence that one site of synthesis is in the tubular portion of this gland, extirpation of the salivary gland from newborn mice has no apparent adverse effect on the development of the sympathetic nervous system in animals. Moreover, extirpation of submaxillary glands from adult male mice causes an initial fall in serum NGF levels, followed in about four weeks by a secondary rise to preoperative levels, again suggesting that other sites of NGF synthesis can be called upon, when required. NGF has also been shown to be synthesized by certain sarcomas of mice. The possible relevance of this to the reported association of ALS with neoplasia and with finding of NGF synthesis by mouse L cells needs to be explored further. While the exact role played by NGF in the normal development of the nervous system is not clear, there is no question that this potent agent has biological significance: 1. The growth factor is present in normal tissues and sera, and is selectively accumulated by its target tissues. 2. NGF has dramatic morphological and metabolic effects on target tissues in vitro and in vivo, and is by far the most potent stimulator of nerve growth discovered to date. 3. Sympathetic nervous tissue is destroyed in vivo and in vitro by antiserum to NGF.

It is believed that studies of NGF may provide model systems with which to approach ALS and other neurological diseases. The reasons for motor nerve cell death in ALS are still obscure. It is reasoned that growth promoting factors may be required by other nerve cells as well as by the sympathetic nervous system, and that if these factors are not elaborated or if they are removed following development within the organism of antibodies to them, systematized degeneration of nervous tissue may eventually ensue. Current work is underway to explore the possibility that ALS occurs because an antiserum directed against a surface component of motor neurons or against a "hormone" which interacts with the neuronal surface, kills them just as anti-nerve growth factor kills sympathetic neurons. It may be that the neurotoxic factor or factors already demonstrated in ALS sera are antibodies to such a surface component.

The following lines of evidence have been developed pertinent to the theory of ALS pathogenesis: 1. There may be a receptor site for mouse salivary gland NGF on the surface of neuroblastoma cells of human origin. 2. Mouse neuroblastoma cells may have NGF receptors in their surface, but do not synthesize NGF. 3. Mouse L cells (transformed fibroblasts) and other fibroblast cell lines synthesize large quantities of NGF. 4. Salivary gland NGF and L cell NGF directly stimulate mouse and human macrophage DNA synthesis and indirectly stimulate lymphocytes.

In view of these observations, a hypothesis is advanced that ALS may result from some derangement in function of a trophic factor related to NGF. Anterior horn cell disease might result from an antiserum directed against a surface component of motor neurons, or against a trophic factor which interacts with

the neuronal surface, just as antiserum to NGF selectively kills nerve cells in sympathetic ganglia. If NGF can be implicated in ALS and if the hypothesis is true that the factors in ALS serum which are toxic to anterior horn cells are antibodies to nerve cell membrane or to a trophic substance akin to NGF, a logical approach to therapy might be developed.

Neurochemistry: A recently published study of brain cortex from two patients with ALS presented evidence that a disturbance in protein metabolism occurs in ALS. This evidence was that in these patients, the total protein content of neuronal cell membranes was markedly reduced, and that an extra band was observed among the heavy molecular weight proteins of myelin.

As part of the effort to explore the etiology of ALS, proteins and subcellular fractions of human brain will be studied. There are two major objectives. First, if there is an immunologic mechanism involved in ALS, this mechanism can only be established by detecting the offending antibody. Such detection will probably require concentration of the appropriate brain antigen, since the immunological methods of detection, in common with all methods, have limitations in sensitivity and specificity, and these limitations can only be overcome by obtaining the appropriate reactive components in higher concentrations. Second, if there should be a viral mechanism involved, then it can be expected, from present knowledge of the effect of viral infections, that some unusual protein component is produced in the infected tissue. Recent advances in techniques for separating and isolating proteins suggest that it will be worthwhile to examine brain tissue from ALS patients in the search for proteins with appropriate immunological or other properties. This applies as well to the study of subcellular fractions. Pilot studies with crude protein fractions of one ALS brain and a synaptosomal fraction from a normal brain have already been shown to be active in stimulating migration inhibitory factor production in lymphocyte culture from some ALS patients. The objective of these studies is to prepare antisera to the purified proteins. Such sera could then be used for immunochemical characterizations of brain proteins both in ALS and other human materials by such techniques as immunoelectrophoresis, gel diffusion and radioimmune assay. Physical and enzymatic characterization of the protein fractions will be undertaken to permit both qualitative and quantitative comparisons among brain samples, with the hope of finding specific changes in ALS brains such as deletions or appearance of new proteins, consistent with either loss of specific cells or cell products or with synthesis of new products such as would be directed by viral genome. The program is to find clues for etiology of disease processes where none exist. The probabilities of success are entirely unpredictable; however, the characterization of human brain proteins must yield information which will certainly be useful. If specific antibody can be produced and labeled, it should provide a powerful tool for localization as well as characterization of brain components, not only in a disease such as ALS, but with blood and far-reaching applications.

Nerve tissue culture studies: Recently, a major advance in our understanding of amyotrophic lateral sclerosis (ALS) has been made by the discovery that diluted sera from ALS patients were toxic to cultured neonatal mouse anterior horn cells. The use of an *in vitro* system not only lends itself to quantitative study but circumvents the blood-brain barrier and permits the direct contact of the test sera with monolayered anterior horn cells. One study has

already shown that a factor in ALS sera may have a specific cytotoxicity on the anterior horn cells, since sera from patients with other neurological disorders (for example, Multiple Sclerosis, Myasthenia Gravis, Werdnig-Hoffman, Guillain-Barre, and Huntington's Chorea) were inactive. The data further indicate a cytotoxic effect solely at the level of the anterior horn cells since the non-neuronal components appear unaffected. This observation will be repeated with a quantitative method for the early evaluation of the ability of the various ALS sera, cerebral-spinal fluid (CSF) and lymphocyte supernatants containing lymphotoxin to inhibit in vitro nerve development. It is proposed to investigate the specificity of response of other neuronal and non-neuronal tissues to ALS sera. In order to see if ALS sera and CSF specifically affect motor horn cells or if other neurons are susceptible, brain slices as well as sensory and sympathetic ganglia will be cultured in the presence of control and ALS sera and CSF. A variety of tissues will be utilized including those of chick, mouse, and human embryonic origin. Although the nature of the cytotoxic factor has not yet been identified, it is known not to be dialyzable and probably proteinaceous. Preliminary work was carried out in coded sera samples from control, ALS and MS patients incorporated into the culture media and applied to established, and freshly prepared embryonic chick dorsal root ganglia. The sera from patients with either ALS or MS significantly inhibited neuronal development when compared to controls under either of the two culture conditions. Pilot experiments using spinal cord cultures with diluted sera from patients with ALS showed a specific cytotoxicity to cultured mouse anterior horn neurons. The effects of sera collected and tested for interferon have been studied on segments of cervical and thoracic spinal cord from 9 day chick embryos. The embryos were dissected under sterile conditions and the control segments were explanted onto collagen coated coverslips in double Maximow preparations after being fed 199 medium supplemented with 40% fetal calf serum and 600 mg/100ml glucose. After several days, the coverslips were transferred to roller drum tubes and maintained with biweekly feedings for four weeks as Roller Drum Cultures, after which they were transferred back to depression slides and treated with the appropriate medium containing the test sera.

It was observed that the sera from the two ALS patients destroyed virtually all of the neuronal networks. Sera from two patients with multiple sclerosis or those of control patients did not exhibit a similar cytotoxic effect on these neuronal networks. These pilot studies suggest that ALS sera may contain a factor not only capable of causing anterior horn cell degeneration of mouse cultures but also of similar chick embryonic cord cultures. These studies further support the suggestions that the use of a nerve culture assay system may provide a valuable tool in the study of the pathogenesis and etiology of ALS. Further experiments are now in progress, including high-resolution time-lapse studies on the development of anterior horn cells and ALS induced degeneration of six week old chick spinal cultures.

Interferon: The rationale of the experiments involving interferon is three-fold: (1) the presence of interferon in the CSF and serum of patients with ALS might serve as circumstantial evidence for the involvement of a virus-like agent. (2) The presence of interferon in cultures of cells inoculated with extracts of kidney or brain tissue or with CSF from ALS patients might indicate the presence of a viral agent in such cultures. (3) As interferon is produced

by sensitized lymphocytes *in vitro* exposed to a specific antigen, its presence in cultures of lymphocytes from ALS patients incubated with nerve and kidney tissue extracts could be used as an indicator of lymphocyte sensitization.

There are many reports on the detection of interferon in clinical specimens collected from patients during acute viral infections. For instance, interferon was detected in the sera and nasal washings of human volunteers inoculated intranasally with A2 influenza virus or in the sera of children who had received live measles virus vaccine. On the other hand, interferon could not be detected in multiple serum samples taken from 34 infectious hepatitis patients at various stages of illness.

Perhaps more relevant to the subject of ALS studies are the reports on the incidence of interferon in the CSF of patients suffering from CNS infections. Interferon was found in CSF of patients with aseptic meningitis of viral etiology. Also, interferon has been reported in CSF taken from children with meningitis caused by Coxsackie or echoviruses and high interferon levels were frequently found in the CSF of children suffering from mumps meningoencephalitis. Interferon was generally absent from the CSF of healthy subjects or patients suffering from infections not involving the CNS. The detection of interferon in the CSF or in the serum of patients suffering from ALS would have to be interpreted with caution. First of all, the nature of the material exerting interferon activity in the routine assay based on the antiviral action in human cells would have to be rigorously characterized. It is now recognized that there are at least two distinct types of interferon: one is made in a variety of somatic cells, including lymphocytes, correlated with the other parameters of cell-mediated immunity and the other type is produced in response to the first inoculation of culture, by virus or even non-viral inducers. These two types can be distinguished by the stability at pH2, stability at 56°C and heterospecific activity in rabbit kidney cells.

Experiments will include the testing of supernatants of lymphocyte cultures, prepared from ALS patients or control individuals, after their exposure to nerve and kidney tissue extracts. The presence of interferon in lymphocyte cultures from ALS patients could be employed as one line of evidence for the state of sensitization of ALS lymphocytes to some antigens present in nerve or kidney tissues. If present, this interferon should have the characteristics of type II interferon. Search for interferon in culture fluids of ALS lymphocytes incubated in CNS and kidney tissue extracts from ALS patients will be made and tested for the presence of interferon along with various supernatant fluids from control lymphocyte cultures. If activity is detected, characterization of the active component would be undertaken. The results of interferon assays will be correlated with the presence of migration inhibitory factor.

Epidemiological studies of ALS in Japan: For the last three years extensive investigations of ALS have been carried out, which occurs in high incidence in two separate foci in the Kii peninsula in Japan. Genetic data have been ambiguous. While in one of the loci there is a high incidence of consanguinity, in the other locus this is not the case. Furthermore, since 1967, there has been a decline in the incidence of the disease in those areas where the disease incidence had been greatest. The inconsistency of genetic data, together with the recent reduction in frequency has led to postulate the presence of an

exogenous factor. It is now postulated that this exogenous factor may relate to secondary hyperparathyroidism. It has been found that in Guam (but not in the Kii peninsula) the drinking water is high in manganese--and that the rivers in the Kii peninsula have low calcium and magnesium levels. Also reported are the elevated levels of parathyroid hormone in 46 percent of ALS cases--compared to 16 percent of controls, and increased calcium and aluminum levels in post-mortem ALS neuronal tissue, studied by neutron activation analysis. It is noted that hamsters fed a calcium and magnesium deficient diet developed changes in the anterior horn cells and had increased calcium levels in the nervous system.

It is now proposed to continue the epidemiological studies, and to continue examination of metal metabolism. These latter studies will include assessment of parathyroid function in ALS cases, and therapeutic trials of calcitonin and vitamin D.

A more targeted issue is the inquiry into a possible relationship between ALS and hyperparathyroidism. Previous work suggests the converse may be true, i.e., that patients with hyperparathyroidism may have anterior horn cell dysfunction. Because of the access to so many ALS patients, it is now possible to answer these questions. This provides additional rationale for the proposal to study calcium metabolism in ALS.

VIRUSES AND NEUROLOGICAL DISORDERS

Earlier workers in virology suspected the possibility that some viruses might remain dormant in the body for a prolonged period before producing overt disease and that others may be responsible for persistent and/or recurrent infection. Most of the clinical and experimental viruses are identifiable by a relatively short incubation period lasting only days or at the most a few weeks, with rapid proliferation of virus prior to the acute phase of disease followed by the disappearance of virus resulting in an increase in specific antibody that is supposed to provide protection against future infection with the same virus. Opposed to rapid onset of viral disease, virus infections with a prolonged incubation period and slow initiation of pathological processes may be responsible for many chronic and degenerative neurological disorders of man. Added evidence to the concept of "slow viruses" was gained when veterinary virologist Sigurdsson identified three infectious diseases that affected native Icelandic sheep not observed anywhere else previously. Two were neurologic and one a pulmonary disease. All had a protracted incubation period which resulted in the animals' death. Since then several other slow viruses both in animals and man have been found which specifically attack the nervous system. This report in general is related to these neurotropic viruses.

Subacute sclerosing panencephalitis (SSPE) is a degenerative neurologic disease of children and young adults. It is characterized by progressive mental and motor deterioration, myoclonic jerks, and coma. The patients become severely emaciated and die from intercurrent infections. The diagnosis established during the incipient stages often shows a personality disorder or mental retardation and the EEG shows slowing and dysrhythmia. However, high amplitude, low frequency synchronous waves do not develop until the patient exhibits myoclonic jerking. Spinal fluid proteins and cell counts remain normal or increase slightly during the entire course of the disease. Transmission of encephalomyelitis from humans to animals and further from animal to animal, producing symptoms typical of SSPE in the animal, has provided an important new lead in isolating and understanding the causative agent in SSPE. During the last few years, evidence of a relationship between SSPE and measles virus has been established.

Measles virus, once considered primarily a respiratory disease agent, has within the last few years been recognized as an agent capable of producing a variety of pathologic states within the CNS. Measles has long been known to produce an acute type of CNS inflammation with subsequent demyelination during so called "post infectious encephalomyelitis," a condition in which the pathogenic mechanisms are still unclear. In 1966, new evidence showed that an agent antigenically identical to measles virus was etiologically involved in the chronic childhood disease SSPE. Convincing serologic, morphologic and virologic information since has shown that each case of SSPE contains a variant of measles virus. Increasingly, serologic data has accumulated suggesting that measles is also etiologically implicated in many cases of multiple sclerosis (MS). To date it has been impossible to isolate measles from MS patients and the pathogenesis of the disease still remains unclear. Nevertheless, such information indicates that measles virus commonly infects the CNS of man and, at least in some cases, remains in latent form to produce remitting or chronic disease months or years after the primary infection.

In order to better understand the pathogenic mechanisms by which measles virus reaches and remains within the CNS, a variety of animal models have been attempted: a subacute disease in ferrets, dogs, calves, and monkeys was produced by inoculating the animals with cells containing the LEC strain of SSPE virus. During 1971 studies were undertaken with a strain of SSPE virus isolated from a human brain biopsy which was later adapted to the hamster CNS. This strain was labeled HBS virus. This virus produced an acute, universally fatal, giant cell encephalitis when inoculated intracerebrally into newborn hamsters but failed to propagate in extramural tissues. Recently, an experimental chronic measles infection of the hamster CNS has been established which bears many of the virologic, histologic, serologic and ultrastructural characteristics of human SSPE. This model is now being used to answer some of the questions posed here: What is the state of the defective CNS virus? May the virus exist in one of several defective conditions? How are measles antigens expressed during the defective infection and do they intermittently appear as in simpler in vitro systems? What is the relationship of known measles antigens to structural viral components such as the "fuzzy" and smooth nucleocapsids? What components of the host immune system appear and what are the relative functions of the humoral and cellular immune systems during chronic infection? Can the latent infection stimulate the immune system to immunopathic destruction of neural tissue?

The exact relationship of SSPE virus to measles virus may be defined only after detailed biochemical and genetic studies. Reports have appeared which describe some chemical and physical properties of measles virus RNA and of RNA and nucleocapsids from measles virus infected cells. The comparisons of virus specified RNA's in measles infected cells with those in SSPE virus infected cells have revealed thus far only quantitative differences among six distinct species of RNA. Studies are underway comparing measles and SSPE viruses in terms of virion RNA and peptides, as well as virus specified peptides of infected cells. Mutants of measles and SSPE virus have been isolated which are temperature sensitive and which have other markers that will serve in both biochemical and genetic investigations.

The molecular biology of measles virus has been neglected until the relatively recent demonstration of the relationship of this virus to SSPE. The ts mutants have been isolated and will serve as valuable tools for studying measles virus replication. Thus far, four out of the possible eight to ten complementation groups of measles virus have been identified and partially characterized. At the nonpermissive temperature some of the ts mutants bear a striking phenotypic resemblance to the viral genome present in SSPE brain or brain cell cultures, i.e., production and accumulation of viral antigen in cytoplasm and nuclei, with or without syncytia formation, and little or no production of hemadsorption or infectious virus. These similarities suggest that a single mutation in the measles virus genome can result in the type of restricted virus replication seen in SSPE.

Nine of the eleven ts mutants examined were attenuated for production of acute encephalopathy in newborn hamsters. None of the ts mutants have produced a "slow" nervous system disease in these animals. The latter observation may reflect the fact that the hamster's body temperature is permissive for these mutants or that other factors, e.g., immunologic response, are important for

production of slow disease. Thus, in searching for an adequate experimental model of SSPE, ts mutants which are restricted at 37°C and others which represent additional complementation groups are being tested in immunologically modified hamsters or in another experimental host.

Previous studies on biological and biochemical characteristics of the SSPE viruses are all compatible with the suggestion that these viruses are variants of classical measles virus. Demonstration of complementation between a ts mutant of SSPE virus and other mutants of classical measles virus is in accord with this suggestion. Comparison of SSPE viruses and several other strains of measles virus as regards their susceptibilities to interferon also indicated that the two groups of viruses are very similar. Demonstration of significant differences between the SSPE strains and classical measles virus requires detailed analysis of viral nucleic acids and polypeptides. These studies will be facilitated by using cycloheximide to induce accumulation of virus specific mRNA from which individual classes may be isolated.

The interferon experiments, especially those using brain cell cultures and infectious center assays, are preliminary to testing the effects of interferon on "nonproducing" SSPE brain cell cultures. Results of the latter tests will be useful when considering possible therapeutic trials of interferon in SSPE patients.

The hypothesis that a mutation in the measles virus genome present in SSPE is primarily responsible for the observed block in virus replication is supported by a variety of observations and can be tested further if the SSPE viral genome can be retrieved from "nonproducing" SSPE brain cells by superinfection with an identifiable helper virus, e.g., a ts mutant of measles virus. Replication of the SSPE virus present in the patients' brain cells is blocked in the same or a similar state in all patients. Genetic or phenotypic definition of the stage of infection which is blocked will be important in elucidating the pathogenesis of SSPE and may also prove to be useful in monitoring the safety of live measles virus vaccine. It should also be noted that, if the hypothesized mutation occurs naturally in wild or vaccine measles virus, SSPE may be an infrequent result of infection of an otherwise normal individual with an "ordinary" strain of measles virus in an environment which is not unusual. Isolation of virus from subacute and chronic nervous system diseases other than SSPE, would have obvious implications for prevention and possible therapy.

Progressive multifocal leukoencephalopathy: PML is a subacute to chronic demyelinating disease of man much rarer than multiple sclerosis, but of worldwide distribution. The disease involves adults only, chiefly during the 5th and 7th decade of life and occurs usually as a late complication of general body malignancies, especially of chronic leukemias and lymphomas, but also of benign diseases of the reticulo-endothelial system. From these background diseases and associated treatments, it was suspected that impaired immunologic responsiveness played an important role in the pathogenesis of PML. As anticipated, the disease has more recently been found in four renal transplant patients with extensive iatrogenic immunosuppression and also in a patient treated for complicated rheumatoid disease.

The early lesions of PML are multiple pin-head sized demyelinated foci usually at the border between cerebral cortex and white matter. Later, these lesions enlarge, coalesce and spread to involve most of the white matter of one or even both hemispheres. A unique cytopathology is the main criterion by which the disease is separated from multiple sclerosis and other demyelinating diseases. The myelin supporting oligodendrocytes are the first glial cells to show nuclear abnormalities and these are pathognomonic: the nuclei are greatly enlarged, the chromatin pattern and its stainability are abnormal, and ill defined inclusions are present. In older lesions most of these cells have lysed, and atypical bizarre giant astrocytes have appeared which resemble the malignant cells of pleomorphic glioblastomas. However, an association with a true astrocytic brain tumor was not noted until 1974 when multiple whitish tumor nodules of 'glioblastomatose en plaques' were seen in the demyelinated white matter of an 18 year-old patient with PML who had a severe problem of the hemopoietic system since age 2 years. Inflammatory infiltrates of the meninges are not seen in PML (the spinal fluid is unremarkable), and mononuclear parenchymal infiltrates are very rare.

The presence of Papova virions was noted in the nuclei of oligodendrocytes of two formalin fixed brains examined in the electron microscope. The virions were present in lesions only, and not in remote areas, or in a control brain fixed in formalin. The papova virus group includes DNA viruses of two different sizes. The only previously known human papova virus, the wart virus, belongs to the larger sized subgroup (45-55 nm). The PML virions, however, from their smaller size (30-45 nm), seemed to belong to the polyoma-SV40 subgroup known chiefly for their oncogenic potential. Such virions had not been seen in any human tissue before. By now, it is almost routine for PML case reports to include confirmation of the diagnosis by electron microscopy. Papova virions are found in human brain tissue exclusively in lesions of PML.

JC virus, a human papova virus: Between the discovery of the papova virions in brain tissue and 1971, repeated attempts were made in different laboratories to culture a "PML" virus in a wide variety of primary and serially passaged cells and in many species of animals, including Rhesus monkeys. In 1971, efforts were successful in isolating and cultivating a virus from the brain of a patient (J.C.) with longstanding Hodgkin's disease. This brain was massively involved by PML and had an overabundance of abnormal oligodendrocytes. Primary human fetal glial cells were used as cultures, for the first time in all these studies. Transmission electron microscopy of cultures fixed 18 days after inoculation showed virions, identical to those seen in the JC brain tissue, in about 50% of glial cells. The isolate was named JC virus, after evidence had been accumulated that the culture propagated virus was indeed derived from the virus in the brain. From comparative data with previously known papova viruses, it was established as a new human papova virus. To date, JC virus has been either isolated or identified by immunofluorescence in brains of six additional cases. It would appear that JC virus is a stable human pathogen.

Serologic evidence has been obtained that JC virus circulates widely in the human population. By 14 years, 60% of children have antibody, by middle age 75% of sera are positive. The portal of entry of the virus is unknown and no illness, aside from PML in the adult, has been linked to the virus. In

younger age groups, the virus might produce an entirely different tissue reaction, and not a degeneration. It might cause an acute mucosal or glandular inflammation, or even a neoplasia. The JC virus, however, is unique in its ability to produce medulloblastomas and pineocytomas when injected intracerebrally, whereas other papova viruses produce ependymomas and plexus papillomas following intracerebral inoculation. In one case, a systemically inoculated female became pregnant and delivered a malformed fetus, raising the possibility of teratogenic effects of JC virus. The most important implication of these findings is that JC virus might play an oncogenic role in human medulloblastomas. Current studies include examining human medulloblastomas for evidence of JC viral antigen and the evolution of tumor formation following intracerebral inoculation of JC virus into newborn hamsters. The viral-host relationship during the induction of tumors and during ontogenetic development of the cerebellum will be examined by light and electron microscopy coupled with immunofluorescent studies of viral T antigen.

As yet, no animal model for PML has been found. Mink, some of them immunosuppressed, and Rhesus monkeys, owl monkeys, and marmosets have not shown disease of the CNS after observation periods from 1/2 - 2 years after inoculation by various routes with JC virus.

A number of viruses have been implicated in the etiology of multiple sclerosis. It is surprising that vaccinia infection, almost universally distributed, has not been sought as a possible agent. However, no studies on vaccinia studies of multiple sclerosis have been done with the exception of electron microscope searches for elementary particles which have been unsuccessful. It is clear that, if vaccinia antibodies are found in a significant number of spinal fluids of multiple sclerosis victims and in no other chronic neurologic disease, this might be of some diagnostic use for neurologists faced with early states of the disorder, such as optic neuritis, which may or may not progress to the full-grown picture of multiple sclerosis. More important, if vaccinia can be related to etiology, a significant reduction in incidence of routine vaccination may lead to a reduction in this disease. A study is being conducted on the frequency of the presence of neutralizing antibodies against vaccinia virus in the spinal fluids of victims of multiple sclerosis and of other patients suffering from chronic brain syndromes. Preliminary evidence suggests that only multiple sclerosis patients have such antibodies in spinal fluid. In order to relate the possible etiologic significance of this finding to the disease itself, it is proposed to do fluorescent antigen studies on brains of multiple sclerosis victims using specific antibodies against four soluble antigens of vaccinia (NP, S, HS, HL).

Visna is the only model of slow, virus-induced demyelinating disease in animals, but work on this infection has been limited because the disease could only be produced in Icelandic sheep. Previous attempts to adapt visna virus to other breeds of sheep or other animals have not been successful. Because of the importance of this model to study demyelination relative to multiple sclerosis, further attempts have been made to adapt the visna virus. Preliminary studies have been carried out using the rapid serial passage of visna virus in fetal sheep. Visna was originally recognized in Iceland as a naturally occurring fetal central nervous system disease of sheep and was described by Sigurdsson as one of the prototypes of slow infection. The

causal agent is now classified as an oncornavirus, based on its physical, biochemical, and biological properties. Following intracerebral inoculation of susceptible Icelandic sheep, a persistent infection develops followed by a late appearance of neutralizing antibodies.

This study postulates that the disease is immunopathological in nature and will attempt to elucidate (A) the cellular sites of virus replication; (B) the role of the immune response (antibody and cell-mediated) in disease production and (C) the mechanisms of virus persistence. Approaches will include (a) a sequential study of infection in Icelandic sheep; (b) the effect of immunosuppression, using antilymphoid serum or cyclophosphamide; (c) the effect of active immunization before and after inoculation; (d) the correlation of various measures of antibody and cell-mediated immunity with disease progression; (e) a search for evidence of an auto-immune response against myelin antigens; and (f) the influence of age of inoculation upon the course of infection.

Major pathogenesis experiments so far conducted indicate that nearly all sheep injected intracerebrally with strain 1514 of visna virus nrvs,r infected over a varying period of time up to 12 months. Virus was frequently present in CNS, spleen, and lymph nodes and less commonly in other sites. All sheep developed pathologic lesions, particularly severe periventricular inflammation. Further evidence of infection was obtained from blood and CSF, complement fixation test and neutralizing antibody responses. These results are encouraging in that the production of visna-mediated CNS disease is more reproducible in frequency and severity than has ever been seen in the past. Much of the future work involving electromicroscopy, immune response and immunosuppression is now being carried out on this reproducible model.

A possible relationship between Experimental Allergic Encephalitis (EAE) and visna virus is being investigated. The pathology of EAE, as described for animals such as the guinea pig and rat bears considerable resemblance to visna in the prominent nature of the inflammatory reaction, its cellular composition, and the subsequent destruction of CNS parenchyma, particularly of the white matter. Furthermore the slowly developing lesions in visna suggested the possibility that sensitization to CNS antigens might be initiated during the prolonged incubation prior to clinical signs. These arguments suggested that the hypothesis of an auto-immune component in visna should be investigated as one aspect of this study. The strategy selected was first to produce EAE in the sheep, and if this proved readily feasible, to set up assays for antibody and cell-mediated responses to basic protein and other CNS antigens. These tests could then be applied to visna-infected animals to search for evidence of an auto-immune phenomenon.

The possibility that visna infection initiates an auto-immune process is being studied by production of experimental allergic encephalitis (EAE). Work to date has documented that acute EAE can be readily induced in sheep, which develop a fulminating and fatal form of disease, a finding not previously described. This observation has stimulated an intensive effort to develop in vitro correlates of the immune response, to quantitate both antibody and cell-mediated immunity.

Results to date indicate that affected sheep have very severe inflammation throughout the CNS, mainly due to infiltrates of lymphocytes and monocytes. Complement fixation antibody tests have been developed, using both basic protein and an alcohol extract of brain. Most sheep show a rising serum titer to both of these antigens following sensitization. Also a considerable proportion have pre-sensitization antibody to basic protein, a result requiring further evaluation. Using basic protein, peripheral blood leukocytes undergo a lymphocyte transformation response when taken from sheep with acute EAE. Application of these tests to visna-infected sheep has so far failed to reveal evidence that there is an antibody response to basic protein during the course of infection. However, the presence of pre-infection basic protein antibody in considerable numbers of sheep and the limited time of follow-up (3-5 months) precludes definite conclusions. Less extensive tests for lymphocyte transformation have so far shown normal indices in visna-infected sheep.

Visna is a slow progressive demyelinating disease of the central nervous system of sheep caused by a virus which shares many biological properties with the RNA tumor virus. The proposed research will examine (a) the mechanisms of persistence of virus, (b) the pathogenesis of the demyelinating lesions in the brain and spinal cord, and (c) the effects of altered host immune competence on the outcome of infection. The study is divided into three parts: short-term studies in infected young adult lambs, long-term studies in the same group of animals, and infection of thymectomized fetal lambs. On the basis of these experiments, studies will be directed to: (a) identification of infected cells using virologic techniques both at the cellular and molecular levels, histochemical tests including immunofluorescence, immunoperoxidase, and autoradiography and electron microscopy, (b) the lymphoproliferative aspect of the disease using virologic and a variety of immunological techniques, (c) the humoral and cellular immune responses to the infection and the pathological effects, or lack of, in infected immunologically compromised lambs.

Initially, visna virus was cultivated in primary sheep choroid plexus cells and primary sheep testis cells following methods of Sigurdsson. A plaque assay and an antibody neutralization assay for the virus as well have demonstrated North American lambs are susceptible to intracerebral infection with the virus. Clinical symptomology and pathological changes are similar to the field disease reported in Iceland. Fetal infection was also accomplished with evidence of persistence of virus in brain and lung for periods up to twelve weeks. Four-week-old lambs yielded virus from brain explants but not from cell-free tissue homogenates. Perivascular changes in the white matter were noted together with a mild, focal meningitis and an interstitial pneumonia. Lymphocytes react to virus in two ways. They were specifically sensitized in vivo to visna virus antigen and responded to antigen with blastogenesis in vitro. Lymph node cells were shown to be infected by visna virus in vitro with subsequent stimulation of DNA synthesis but without significant replication of virus.

Investigations are being conducted to understand the intricacies surrounding the replication of visna virus nucleic acid species and how these synthetic processes relate to the ability of this slow virus to persist in susceptible

host tissue until overt clinical symptoms appear. The formation of visna virus DNA intermediates designated as "DNA provirus" will be studied in cultures of sheep choroid plexus cells, and the presence of these virus-specific DNAs will be detected by testing the DNA fraction of infected cells for its capacity to stimulate reassociation kinetics of denatured DNA generated in vitro by reverse transcriptase or by testing for its integration within "network" DNA formed by Britten-Kihn repetitive sequences under conditions of self-annealing. The aim in these experiments is to determine whether visna "provirus DNA" occupies a specific site on a host chromosome and associates with a given size class of cellular DNA. The effect of various inhibitors of protein and nucleic acid synthesis on the formation and integration of these visna DNA species will also be examined.

Other studies planned are molecular investigations of viral-specific nucleic acids and proteins in diseased tissue of sheep infected with visna virus and a search for resident gene products of covert slow virus genomes in human autopsy specimens of brain and other organs from victims of multiple sclerosis. The latter tests will focus on demonstrating viral nucleic acids having sequences homologous to visna virus by reassociation kinetics of visna transcriptase product, by DNA-RNA hybridization studies with labeled visna RNA and by use of complementary "minus" strand DNA generated by the visna transcriptase for implicating the presence of viral RNA transcripts in cell fractions.

Ultrastructural investigation of viral infections involving the central nervous system of experimental animals and man is being conducted. Emphasis is being placed on disorders in which a viral infection may be associated with demyelination or with a teratogenic effect. Human biopsy material from chronic degenerative disorders of the CNS of suspected viral etiology will also be screened electron microscopically for detection of a possible agent. Specifically, cerebellar malformation induced by the Kilham rat virus, and the effect of the blue tongue virus on the cerebral cortical development will be examined. The study of the demyelinating disease induced in mice with the JHM strain of mouse hepatitis virus will focus on two specific questions; namely, the mechanism of demyelination and the role of the immune response in demyelination, and the source of cells involved in remyelination. Human studies will consist of a continued attempt to determine the occurrence and frequency of viral isolates in progressive multifocal leukoencephalopathy, and electron microscopic monitoring of cultures derived from patients with known and suspected viral disease of the nervous system.

The objective of the multiple sclerosis (MS) virology program is to isolate infectious agents from tissue obtained from patients with multiple sclerosis and to characterize them and determine their relationship to the disease. The rationale for a virologic approach to the study of multiple sclerosis is based on the fact that some clinical features are common to both multiple sclerosis and the known slow virus diseases. Serologic and epidemiologic information incriminate a viral agent as the cause of multiple sclerosis. The recent isolation of a virus (6/94) from the brains of two patients with multiple sclerosis lends further support to the viral etiology concept. The multidisciplinary approach to the MS problem (clinical, virological, immunological), combined with the newer laboratory techniques (cell fusion, use of halogenated pyrimidines, immunosuppression) to activate viruses in tissue

culture and in experimental animals, offers a better chance for the discovery of a virus than has previously existed.

Multiple sclerosis fits the three criteria established by Sigurdsson for slow virus diseases (a) after infection, a period of many months or years without signs of disease; (b) after clinical signs appear, a protracted course, usually ending in serious impairment or death; (c) infection limited to a single host species and lesions limited to a single organ system. Thus, MS is probably acquired but not manifested during childhood, runs a protracted course, seems to be species specific for man and attacks initially only the CNS.

Some of the known slow virus diseases of man (kuru, Jakob-Creutzfeldt and SSPE) and animals (scrapie, visna, transmissible mink encephalopathy and Aleutian disease of mink) have been shown to be transmissible to susceptible hosts. Although several studies have been conducted to induce or propagate these viruses with MS-tissue extracts, the efforts so far have remained unsuccessful. On the other hand, inoculation of animals with cells or tissue extracts obtained from cases of SSPE, progressive multifocal leukoencephalopathy, kuru, Jakob-Creutzfeldt, mink encephalopathy, scrapie and visna resulted in the production of disease. Only with two viruses, SSPE and PML, was it possible to demonstrate and characterize a virus or its components inside CNS cells. With tissue obtained from Jakob-Creutzfeldt cases, it has been possible to relate the presence of a virus to morphological transformation in both the original brain cultures and in cells derived from cultures exposed to the original brain cells.

Recently, after brain cells from two cases of MS were fused with indicator cells, an agent referred to as the 6/94 virus was isolated. This agent has now been propagated in embryonated hens' eggs. Hemagglutination-inhibition tests with egg-grown 6/94 virus permitted it to be differentiated from both the HA-2 and Sendai agents; two prototypes of parainfluenza viruses to which 6/94 is related. Using physico-chemical techniques such as purification, determination of molecular weight, sedimentation co-efficient, analysis of RNA species and electrophoretically-distinguishable protein migration, 6/94 virus has been further studied and compared with the related Sendai virus. The 6/94 virus inoculated intracerebrally into newborn Syrian hamsters caused severe clinical and pathological syndrome. On the other hand, HA-2 virus produced severe hydrocephalus but failed to cause the marked wasting characteristic of 6/94.

6/94 virus has been analyzed for its antigenic, RNA and polypeptide compositions and for selective biological properties in animals and cell cultures. The results establish that 6/94 virus contains a 50S RNA genome and is related to Sendai virus in its antigenic and total polypeptide compositions. Despite these similarities, the 6/94 and Sendai viruses differ in certain phenotypic properties. 6/94 virus is markedly less cytotoxic for chick fibroblasts, especially at 37°C and after β -propiolactone inactivation possesses a greater capacity for cell fusion and a lower toxicity than does comparably-treated Sendai virus. In addition, 6/94 virus shows greater hemolytic activity. Polypeptide analysis of egg grown 6/94 virus and Sendai virus on SDS-polyacrylamide gels revealed that each virion possessed at least 8 polypeptides.

The major polypeptide associated with each of the viruses (M.W. 60,000 daltons) was found to be associated with the nucleoprotein.

It has been pointed out earlier that, in two chronic CNS diseases, visna and PML, a link has been suggested between slow virus infection and viral oncogenesis. The rationale in searching for a similar correlation in MS is supported by the observation that the 6/94 agent is different antigenically, biochemically and biologically from more than one of the parainfluenza I prototype viruses and argues against it being a laboratory contaminant. Also, the presence of nucleocapsids in brain cells of one MS case before fusion, the appearance of virions upon fusion of these cells with both indicator cells (CV-1 and WI38) and lack of virions and nucleocapsids in non-MS brain "fused cultures" argue against the indicator cells being the source of virus. The isolation of a similar (6/94) virus from the second case of MS lends further strength to the possible association of this agent with multiple sclerosis.

Brain tissues obtained at the time of biopsy or autopsy are being studied by light and electron microscopy in order to document and define any morphologic alterations and to search for viruses in tissue sections from patients with multiple sclerosis. These studies also include electron microscopy of tissue culture cells derived from nervous system of patients with MS, EM of supernatant fluid from cell cultures to search for viral particles, morphological characterization of virions, morphological analysis of tissue to define unitype cultures and morphological characterization of 6/94 virus to augment the viral isolation studies and to define virus nucleocapsid in cells prior to fusion and before other tests for the presence of virus could be considered positive.

In general these programs are designed to achieve the following objectives (a) to obtain a viable MS tissue; (b) to investigate a variety of environmental parameters in search for the optimum conditions for the growth of human central nervous tissue in cell culture and their characterization; (c) to continue attempts to isolate, rescue and identify virus(es) from tissue cultured cells using standard and newer virologic techniques; (d) to further characterize the 6/94 virus; and (e) to study immunological reactions in MS patients.

During the past four decades, an obscure disease of sheep called "scrapie" has emerged as an important area of biochemical research. Transmission studies and neuropathological examination of brain tissue suggest that scrapie is a prototype for the spongiform encephalopathies of man, i.e. kuru and Creutzfeldt-Jakob disease. Prior to the transmission of the kuru and Creutzfeldt-Jakob diseases to chimpanzees by intracerebral inoculation of infected brain both of these diseases were classified as "degenerative" abiotrophies of the nervous system.

To date the chemical nature of the scrapie agent remains obscure. The unusual physico-chemical properties of the scrapie agent and its slow replication in the absence of any detection by host defense mechanisms suggest that scrapie is a novel infectious entity. Unlike viruses, the scrapie agent cannot be visualized by electron microscopy and its presence does not provide a detectable immunological response.

This research program is directed toward understanding the chemical structure of the scrapie agent. Because the present assay for scrapie requires determination of an endpoint by titration in mice over 9 months, it is planned to devote effort to the search for a more rapid and less expensive biological assay. A reliable method for the partial purification of the scrapie agent is planned as well as the use of this preparation to explore chemical and immunological assay systems. The purification and subsequent elucidation of the chemical structure of the scrapie agent promises to bring new concepts and techniques to several areas of molecular biology and medicine.

Vesicular stomatitis virus: In conventional acute viral infections, an infected cell dies 8-48 hours after it is infected. In slow-virus infection, virus persists in cells and replicates without causing the death of the host cell. How is it possible that some viruses, even conventional viruses usually associated with cytotoxic infection, persist in cells, replicate and yet not result in cell death? In an effort to understand the mechanism(s) involved in persistence of virus infection, investigators have turned increasingly in the last 10-15 years to in vitro carrier cell cultures. Such cell cultures, through a series of manipulations, become persistently infected with virus. These studies have suggested a number of mechanisms which result in in vitro establishment of persistently infected cell cultures even when highly cytotoxic viruses are employed. Among the mechanisms proposed to account for persistent infection in vitro, two have received considerable attention. The first is the role of temperature-sensitive (ts) mutants in persistent infection; and the second is the role of defective-interfering (DI) particles. While there has been a considerable amount of literature developed over the last 10 years on the roles of ts mutants and DI particles in persistent infection of cell cultures in vitro, there has only been a limited number of studies directed at determining what role ts mutants and DI particles play in vivo in slowly progressive viral disease and persistent infection.

Recently, attention has been centered on studying the in vivo capacity of DI particles and ts mutants of vesicular stomatitis virus (VSV) to induce an altered disease pattern in the central nervous system of mice. Wild-type VSV injected intracerebrally produces a rapidly fatal encephalitis with death of mice in 2-3 days. In contrast, intracerebral injection of mice with DI particles and wild-type VSV, or ts 22 or ts 31 mutants of VSV alone results in dramatically different clinical disease. These mice develop a more slowly progressive CNS disease characterized by wasting and hind limb paralysis. Furthermore, these mice die in 7-10 days rather than the usual 2-3 days seen in wild-type VSV infection. Injection of other ts mutants, ts 11 and ts 41, results in no illness. Additionally, investigators have observed unique, and previously unreported, histopathological lesions in mice infected with ts 22 or ts 31 mutants of VSV. These lesions consist of marked spongiform changes in spinal cords and ependymal area of the brain. Mice infected with wild-type VSV or ts 11 or ts 41 show none of these histopathological lesions. Because of these observations it is proposed to employ certain ts mutants and/or DI particles of VSV to establish a slowly progressive viral disease instead of the rapidly fatal, highly cytotoxic infection usually induced by VSV. The present study is directed towards: (1) defining the in vivo capacity of certain ts mutants of VSV to produce slowly progressive CNS disease and spongiform degeneration; (2) to explore RNA and viral protein synthesis in

these ts mutants as they may relate to the observed histopathological lesions; (3) to define in vivo the capacity of homologous DI particles and either wild-type virus or ts mutants of VSV to produce slowly progressive CNS disease and spongiform degeneration.

Herpes simplex virus (HSV): HSV infection of peripheral autonomic nervous system (PANS) and human disease: It has recently been reported that latent infection of autonomic ganglia with HSV can readily be established in experimental animals. This observation, coupled with reports of HSV in organs innervated by autonomic nerves or in secretory products of these organs raises the question of whether latent and reactivated infection of autonomic ganglia may participate in the production of human disease as well as in the transmission of virus to human contacts. Involvement of local autonomic ganglia in infection of the female genital tract of mice suggests that these ganglia may be important in the passage of virus from the infected human mother to the fetus or newborn with resultant neonatal herpes. Autonomic ganglia, like sensory ganglia, may therefore serve as important reservoirs of latent virus which, when reactivated, may result in significant human disease.

HSV infection of the PANS and neural cell interaction: Despite recent advances in clinical recognition and definition of diseases of the autonomic nervous system in man, studies concerning the etiology and pathogenesis of autonomic disorders are few and understanding remains fragmentary. Investigation of such disease has failed to keep pace with the burgeoning knowledge of the basic biology of the autonomic nervous system. In particular, the question of whether viruses can infect these ganglia and cause clinical disease has received little attention, and, to date, only a limited number of observations of viral infection of autonomic ganglia either in animals or in man have been reported. It is speculated that viral infection of the autonomic nervous system in man may occur more commonly than previously suspected, and that such infection, either by destroying ganglia, by disrupting autonomic neural activity, or by inducing secondary viral infection within innervated target organs, could lead to acute, intermittent or chronic disease in man.

The murine model of HSV infection of the SCG allows experimental testing of this hypothesis. The events leading to acquisition of ganglionic infection, the effects of the infection on the functional and structural integrity of ganglionic neurons, and the target organ sequelae of acute and reactivated infection can all be explored using HSV as a prototype virus.

The more severe human afflictions caused by HSV can be looked upon largely as diseases of the nervous system. Involvement of the central nervous system (encephalitis) in adults or neonates is a severe affliction leading to death or extensive morbidity. Recurrent epithelial eruptions can perhaps also be considered as manifestations of reactivated infection of sensory (and perhaps autonomic) ganglia. Also characteristic of these infections is their variability; infection may be lytic, latent or reactivated.

Despite recent advances in the biochemical characterization of lytic HSV infection and the development of models of latent sensory ganglion infection, the factors determining whether infection of neural tissue is to be lytic, latent or reactivated, i.e., the factors controlling neural cell permissiveness,

are poorly understood. Furthermore, using present methods, the cellular basis of latency and reactivation has been experimentally inaccessible.

Because of the extensive background knowledge concerning the anatomy, physiology, chemistry and pharmacology of the PANS, the use of autonomic ganglia to study infection of neural tissue with HSV presents a unique opportunity. These ganglia are readily manipulated surgically or pharmacologically, their metabolic and functional profile can be followed with well characterized biochemical markers, and their in vitro cultivation has reached an advanced state of sophistication. Infection of autonomic ganglia, therefore, can profitably be exploited to study the factors which underlie alterations in permissiveness of neural cells for HSV. The results of such studies might be applied to other neural cell populations just as studies of development of these ganglia, of their neuronal circuitry and of their synaptic chemistry have been used as models of different and more complex neural structures.

The goal of this project is to define the pathogenesis of latent and reactivated herpes simplex virus infection of the peripheral autonomic nervous system. Among the aspects of this infection to be studied are: (a) the factors leading to acquisition of autonomic infection, (b) the determinants of latency, (c) the cellular changes responsible for viral reactivation, (d) the neural and target organ sequelae of reactivation, and (e) whether latent infection of autonomic ganglia occurs in man. Three complimentary approaches will be employed to achieve the proposed objective: (1) Studies employing murine model of in vivo infection of the superior cervical ganglion of the sympathetic division of the autonomic nervous system, (2) Studies employing an in vitro model of latent and reactivated HSV infection to be developed using dissociated cell cultures derived from autonomic ganglia, and (3) Explantation of human autonomic ganglia obtained at the time of postmortem examination.

These studies will be undertaken because of their direct importance to human disease, because infection with HSV serves as a paradigm of viral infection of the autonomic nervous system, and because they open a new approach to studying infection of the nervous system with this virus.

PARKINSON'S DISEASE

Parkinson's disease is a progressive neurological disorder of unknown cause affecting certain brain areas responsible for the control and regulation of movement. Estimates of prevalence range from one case per 1,000 to one per 200. Primarily a disorder of middle age or later, its prevalence is thought to be increasing with the increase in the average life span.

The onset of parkinsonism is ultimately characterized by tremor, rigidity, and bent posture. Intelligence is usually unaffected. In the fully developed disorder the face becomes the characteristic "mask" with a hard stare coming from unblinking eyes. The patients often sit motionless, rarely crossing his legs or folding his arms.

Standard treatment in the past consisted of physical therapy, a variety of drugs, and surgery. The treatments of choice are now thalamic surgery or a highly effective form of replacement therapy making use of large oral doses of L-DOPA. Although L-DOPA induces remarkable improvement in most parkinsonism patients, it has very unpleasant side effects and some patients do not respond. Therefore, a still better treatment must be found and the greatest long range contribution of the research on L-DOPA may be that, as the first direct lead to the real cause of parkinsonism, it provides an approach to the development of a much more effective form of therapy. Despite many recent developments, evidence indicates that the newer methods of therapy have not substantially increased life expectancy.

Recently numerous biochemical abnormalities in Parkinson's disease have been found. Most of these findings are related to a decrease in dopamine concentration in the parkinsonian brain. Most of the biochemical changes in dopamine metabolism in the parkinsonian brain can be duplicated by making a lesion in the substantia nigra in animals. The changes in acetylcholine metabolism in Parkinson's disease, reflected by the hypersensitivity to cholinergic stimulation and therapeutic improvement with anticholinergic drugs, also is secondary to the loss of nigro-striatal neurons. The degeneration of the substantia nigra cells and certain other neurons appears to be the primary changes in this disease. Most investigators agree that it is only the neurons which contain melanin (e.g., substantia nigra, locus coeruleus, dorsal nucleus of the vagus, etc.) which degenerate in Parkinson's disease. This suggests that there is some peculiarity of the neuromelanin-containing cells of the central nervous system which predispose them to degeneration in parkinsonism. Investigation of the structure, mode of synthesis and possible function of neuromelanin is one important approach to the etiology and pathogenesis of idiopathic Parkinson's disease. Attempts are being made to determine if there is any biochemical inter-relationship between substantia nigra melanization and the abnormalities in catecholamine metabolism in this disorder.

The aim is to develop an understanding and prevention of such unwanted effects of L-DOPA therapy as drug-induced psychotic behavior and involuntary movements. The hope is to separate these actions of L-DOPA from the therapeutic action against Parkinson's disease. It is also hoped that these studies and similar ones to be performed on psychotic patients will provide new forms of therapy for naturally occurring psychoses as well as those induced by L-DOPA.

An attempt is being made to control psychotic behavior and dyskinesia with cholinergic drugs such as physostigmine, oxotremorine or piperidine while at the same time not interfering with the control of Parkinsonian symptoms by L-DOPA.

The preclinical work includes toxicological studies of the agents used and tests of the agents on various animal models. These models include modification of the ability of L-DOPA to induce turning behavior (toward the side of the lesion) in caudectomized mice and actions of cholinergic drugs on caudectomized mice. Since cholinergic drugs modify the behavior of mice intoxicated by L-DOPA, other intoxications will also be studied such as those of LSD and amphetamine.

Synthesis and pharmacological investigations of dopamine analogs: A series of N,N-dialkyl dopamine derivatives have been synthesized which exhibit dopaminergic actions in animal model studies designed to demonstrate stimulation of the nigrostriatal system. The potency of these analogs varied, depending on the length of the alkyl substituents. There is claimed to be a good correlation between the potency of these compounds in producing dopaminergic striatal stimulation and their ability to stimulate the adenylate cyclase system in mouse caudate homogenates. It is claimed that the stimulation by apomorphine, N-propylnorapomorphine (NPA), and the tertiary dopamine analogs of adenylate cyclase is reduced at higher concentrations, and that this is reminiscent of the failure of these compounds to sustain antiparkinson activity with time. The "autoinhibition" effect may thus be an in vitro index of the tendency of these compounds to exhibit tachyphylaxis with respect to their antiparkinson effects.

Additional N,N-dialkyl dopamine analogs will be synthesized, tested in animal studies for antiparkinson effects, and their antiparkinson properties will be correlated with their actions on caudate adenylate cyclase. It is hoped that the most promising compounds may be useful in the treatment of Parkinson's disease, possibly as adjunctive therapy with levodopa. The levels of these compounds will be assayed in plasma and animal tissues following development of a coupled radiometric enzymatic assay for catechol derivatives, employing catechol-D-methyltransferase and labeled S-adenosyl methionine.

It is speculated that the reduced stimulation of adenylate cyclase and the tachyphylaxis associated with these putative dopamine agonists, in high concentrations or after prolonged administration, respectively, may result from formation of the O-methylated derivatives of these analogs, which may be much less active agonists. O-methylated - N,N-dialkyl dopamine derivatives will be synthesized to test their activities in the animal model and enzyme systems.

A series of experiments are planned to synthesize ³H-labeled N-methyl-1,2,3,4-tetrahydroisoquinoline and study its pharmacokinetics and tissue distribution in mice after administration. The basis for these studies is to examine this apomorphine "molecular segment," which exhibits cholinergic properties, to determine whether it could account for some of the antagonistic effects observed between apomorphine and dopamine.

The rationale for these investigations is that the dopaminergic, antiparkinson effects of apomorphine and dopamine analogs are due to the catecholethylamine portion of these molecules and the antidopaminergic effects of apomorphine and other aporphines due to the piperidine moiety. N,N-dialkyl dopamine derivatives will be synthesized, and animal studies of toxicity and efficacy in animal antiparkinson model systems will be performed in order to select new, promising antiparkinson agents for clinical trials. Preliminary studies suggest that this may be a fruitful avenue.

The synthesis and testing of dopamine analogs for possible therapeutic use may yield new and useful agents. The temporal relations between the development of tachyphylaxis and the expected rate of O-methylation of catechols makes the hypothesis that the former is due to O-methylated derivatives unlikely. Studies of the absorption, distribution and kinetics of disposition of these compounds are being investigated.

Modification of cerebral actions of drugs by somatostatin: Experiments are planned to explore in detail the effect of somatostatin on the uptake of apomorphine and other dopaminergic compounds in the brain in mice. Also tested will be the effects of somatostatin on the behavioral actions of dopaminergic agents in rats with unilateral lesions of the substantia nigra. Different doses and time courses of administration of somatostatin will be used. The reason for undertaking the experiments is the observation in pilot experiments that growth hormone increases, and somatostatin decreases brain uptake of (radioactive) apomorphine. In addition, treatment of mice with bovine growth hormone increases the entry of DOPA into the brain and the behavioral effects of dopaminergic drugs. If the work in rodents continues to be promising, the effects of somatostatin in humans with parkinsonism will be investigated to see if this hypothalamic hormone eliminated some of the long-term complications of L-DOPA therapy.

The possibility that growth hormone may increase the penetration of dopaminergic compounds into brain is of great theoretical and practical interest. Somatostatin inhibits growth hormone secretion and apparently has actions of its own on the central nervous system. It would be interesting to know if any effects of somatostatin were due to inhibition of growth hormone secretion or due to a direct effect of somatostatin. Thus, the studies could provide information of great importance in terms of understanding of effects of peptide and protein hormones on the brain, and in terms of practical management of patients with Parkinson's disease.

Experiments with rats having unilateral lesions of the substantia nigra: In these studies rats will be subjected to unilateral lesioning in the substantia nigra by local injection of 6-hydroxydopamine. After three to four weeks the rats will be examined in automatically recording rotameters, and the ability of various drugs to induce rotational behavior will be determined. Super-sensitivity of dopamine receptors on the lesioned side is expected. Thus, dopamine agonists should differentially activate dopamine receptors on the lesioned side, and the animals should rotate in the direction opposite to the lesion. In contrast, dopamine-releasing agents will produce rotation of the rats toward the lesioned side, since the normal nigrostriatal pathway will be differentially activated. This system is, therefore, well suited to indicate

the nature of the action of agents which stimulate the dopamine receptors either directly or indirectly.

Clinical studies: Cyproheptadine is a serotonin antagonist which enters the brain. Since there have been numerous claims that serotonergic mechanisms are distributed in parkinsonism, and that certain adverse reactions to levodopa are associated with altered serotonin transmission, it is of interest to study the action of cyproheptadine in extrapyramidal diseases. Such studies would, in particular, be relevant to dyskinetic disorders, and the increased output of growth hormone which may be induced by levodopa.

The methods of study to be employed include double-blind evaluation of neurological deficits by means of arbitrarily defined clinical scoring protocols previously found to be effective in a number of studies of this kind.

Dopaminergic agonists have been shown to be therapeutically active in Parkinsonism. On theoretical grounds they should prove superior to levodopa, since (1) they do not require dopa decarboxylase (an enzyme which is depleted in the brain of Parkinsonian patients); (2) they do not form potentially toxic metabolites, such as epinephrine and norepinephrine, and (3) it may prove possible to develop molecules with relatively specific affinity for those striatal synapses which are defective in Parkinsonism, thus allowing a selective increase in the wanted, as opposed to the adverse reactions of therapy.

This approach may be regarded as especially important in the quest for reduction of "on-off" reactions, but in addition, it is of interest to study the neuroendocrinological effects on the pituitary and the possibility of pharmacological interactions with levodopa. The drug involved will be the dopaminergic agonist N-propylnorapomorphine or other congeners found to be active in screening tests on animal models of Parkinsonism.

Biogenic amines and extrapyramidal disorders: Studies are aimed at correlating biochemical (biogenic amines) parameters with neurological abnormalities associated with extrapyramidal disorders. In these studies monkeys with surgically induced lesions in specific brain areas who exhibit abnormal movements are used as an experimental model. The effect of the lesions on putative neurotransmitters is being evaluated. Histological changes resulting from the destruction of various anatomic pathways are to be correlated with biochemical changes. The clinical studies involve an evaluation of the effectiveness of L-DOPA therapy and an investigation of the fate of L-DOPA in patients undergoing treatment for extrapyramidal disorders.

Development of a primate model of parkinsonism producing tremor and hypokinesia, has been an important feature of this project. The administration of L-DOPA in combination with l-alpha-methyl-dopa hydrazine (MK-486), a dopa-decarboxylase inhibitor that acts peripherally, resulted in a transient disappearance of tremor in the extremities contralateral to the lesion with a concomitant development of involuntary movement. Trivastal (ET-495), an agent that stimulates dopamine receptors, had an effect similar to that of L-DOPA. Trivastal relieved the tremor and induced involuntary movements for a longer time than L-DOPA. 2-Br-alpha-ergocryptine (CB 154), another agent

that stimulates dopamine receptors, also has tremor relieving and involuntary movement inducing activities.

The effects of activation and blockade of dopamine receptors on dopamine synthesis in striatal slices show that apomorphine, an agent that stimulates dopamine receptors, inhibits the biosynthesis of ^{14}C -dopamine from ^{14}C -tyrosine more effectively in striatal slices than tyrosine hydroxylase activity in vitro. The effective inhibition of ^{14}C -dopamine biosynthesis in striatal slices by apomorphine could either be due to its accumulation in the dopamine containing neurons and subsequent inhibition of tyrosine hydroxylase or to dopamine receptor stimulating activity of the drug resulting in a feedback control of dopamine biosynthesis. In order to study further the effects of activation or blockade of dopamine receptors on presynaptic dopamine synthesis the effects of Trivastal and of Haloperidol on ^{14}C -dopamine synthesis from ^{14}C -tyrosine in striatal slices is being investigated. Trivastal stimulates dopamine receptors, but unlike apomorphine, does not contain a catechol group and does not inhibit tyrosine hydroxylase activity in vitro. The administration of Trivastal results in an inhibition while administration of Haloperidol results in the stimulation of ^{14}C -dopamine synthesis in striatal slices. Treatment of rats with Haloperidol antagonized the Trivastal-induced inhibition of ^{14}C -dopamine synthesis. These results support the idea that a receptor mediated feedback exists which controls the rate of dopamine synthesis.

The effects of dopamine agonists on tremor in monkeys with ventromedial tegmental (VMT) lesions were investigated. The finding that some drugs which inhibit prolactin secretion have the property of stimulating dopamine receptors was fundamental in screening for new antiparkinsonian agents. Trivastal, 2-Br-a-ergocryptine (CB-154) and Lergotril inhibit prolactin secretion and all three compounds presumably stimulate dopamine receptors. These compounds therefore were tested for their antiparkinsonian efficacy in monkeys with VMT lesions and subsequently clinical studies have been initiated in parkinsonian patients..

Trivastal: The single administration of Trivastal resulted in a dose-dependent relief of the spontaneous tremor in monkeys with VMT lesions. At a dose of 3 mg/kg (i.v.) the tremor disappeared for 3-4 hours and the intensity of the tremor was diminished for an additional 2 hours. The administration of Trivastal resulted also in the development of involuntary movements. These results indicate that tremor and involuntary movements are associated with a common mechanism and that the activity of the dopamine receptors are involved in the regulation of these dysfunctions.

Lergotril: The single administration of Lergotril resulted in a dose-dependent relief of the spontaneous tremor in monkeys with VMT lesions. Following a single administration of Lergotril at a dose of 5 mg/kg (i.p.) the tremor disappeared for 1-2 hours and the intensity of the tremor was diminished for 70 hours. Repeated administration of Lergotril reduced its anti-tremor effectiveness. Following the third administration of Lergotril (third day of treatment) the spontaneous tremor was only relieved for 3-5 hours but did not completely disappear. Sedation and compulsive circling behavior towards the denervated side, but no chorea-type movements, were observed in all monkeys following a single administration of Lergotril. No

sedation or chorea-type movements were observed following repeated administration of the drug.

CB-154: The administration of CB-154 (6 mg/kg, i.p.) resulted in the disappearance of the tremor for 1-27 hours. Repeated administration of CB-154 (5-8 mg/kg, i.p.) resulted in the disappearance of the tremor for at least 48 hours. After cessation of treatment the intensity of the tremor was diminished for another 24-48 hours. The repeated administration of CB-154 resulted in the development of involuntary movements. However, the involuntary movements were of moderate intensity and of shorter duration than the drug-induced relief of the tremor. Only one of the monkeys with spontaneous tremor developed unilateral chorea-like movements following repeated administration of the drug. The data suggest that CB-154 could be a powerful anti-tremor drug in parkinsonian patients with unusually long-lasting actions. In this respect it has considerable advantages over DOPA and ET-495, although the latter compound also possesses relatively long-lasting actions. Furthermore, the involuntary movements are only of moderate intensity after CB-154 and have not been seen for more than a day, whereas the anti-tremor activity can last for 4-5 days.

In clinical studies thirteen patients with Parkinson disease were treated with Lergotrile and an overall improvement was observed in some patients. Alleviation of tremor appeared to be the main clinical feature improved and reached statistical significance. In a subgroup of four patients treatment with a higher dose of Lergotrile resulted in further improvement in all cardinal signs but only the improvement in tremor was statistically significant. Adverse effects included orthostatic hypotension, behavioral alterations and gastrointestinal disturbances, but were severe enough to require drug withdrawal in only one patient. Involuntary movements that could be related to Lergotrile were not observed.

The antiparkinsonian efficacy of bromocriptine: Eleven patients with Parkinson's disease were treated with bromocriptine. Clinically obvious improvement was noted in one or more of the cardinal signs of the disease in six ("responders"). For this group improvement in rigidity and bradykinesia was statistically significant while improvement in tremor though impressive in some patients was, for the group as a whole, not statistically significant. Diurnal fluctuations in performance present in two of the responders prior to initiation of bromocriptine decreased in both. No obvious improvement in any of the cardinal signs was noted in the remaining five patients "non-responders". Clinically the "responders" were older, more severely affected and had been on a higher dose of levodopa. However, they had had the disease for a shorter period of time. It is suggested that failure to respond to bromocriptine may be related to a decrease in the sensitivity of post-synaptic dopaminergic receptors.

L-DOPA and brain serotonin: L-Dihydroxyphenylalanine (L-DOPA), administered in high doses, produces bizarre alterations in human and in animal behaviors. Reported side effects of L-DOPA therapy include: insomnia, depression, hypomania and paranoid reactions. Alterations in animal behavior include psychomotor excitement, aggression, stereotyped behavior and hypersexuality. The aim of this research is to more clearly define the neurochemical mechanism

of these behavioral changes induced by excessive brain DOPA concentrations, in an attempt to understand the behavioral side effects of L-DOPA therapy in human disease.

L-DOPA produces increased dopamine levels in the brain but it also may acutely release serotonin and then inhibit its synthesis. Many of the behavioral alterations produced by DOPA may be due to function or metabolic alteration in serotonergic neurons, and the main emphasis of the proposed research will be to study this relationship. Drugs which block serotonergic receptors (Methysergid-2-bromo-LSD, and cyproheptadine) or deplete serotonin, p-chloro-phenylalanine (p-CPA) will be used as pharmacological tools to study the role of serotonin in the effects of L-DOPA. Reduction of certain L-DOPA behavioral effects by these compounds would indicate indirect (non-dopamine) mediated behavior. (Locomotor activity depression acutely and enhancement when 5-HT is depleted). Behavioral analyses would be complemented by biochemical analyses of L-DOPA influence on serotonin, dopamine and norepinephrine turnover rates and concentrations in specific brain areas.

The long-term goal of the proposed research is to determine the neurochemical mechanisms through which non-physiological body loads of the catecholamine precursor L-DOPA produce biphasic actions on certain animal behaviors. The specific objectives include: (1) determination of the mechanism(s) and site(s) by which low doses of L-DOPA (320 mg/kg, i.p.) reduce spontaneous locomotor activity of male mice; (2) determination of the mechanism(s) and site(s) by which high doses of L-DOPA (560 mg/kg, i.p.) enhance spontaneous locomotor activity; (3) studies to determine if tolerance develops to either or both of the above effects of L-DOPA and elucidation of neurochemical mechanisms by which such a tolerance would be produced. While the basic behavioral measurement to be made in this research is spontaneous locomotor activity in male mice, it is felt that useful generalizations about the mechanisms and sites of action of L-DOPA can be drawn only if these hypotheses are tested in a few other well defined behaviors.

The effects of L-DOPA and D-DOPA, administered both acutely and for chronic periods are being determined on brain amines. The biochemical experiments were conducted in conjunction with experiments on depression of spontaneous locomotor activity produced by L-DOPA and D-DOPA. Many pharmacological treatments were used to enhance and inhibit the depression of locomotor activity. The effect of D-DOPA on motor activity is explored since it is believed that this compound would serve as a non-metabolized control for L-DOPA. D-DOPA depressed the spontaneous locomotor activity of male mice. It also depressed the levels of serotonin which could be assayed in whole brain samples. Dopamine was slightly but significantly elevated by D-DOPA. The mechanism of this elevation of dopamine after D-DOPA treatment remains in question but it does not appear to be attributable to L-DOPA contained in the D-DOPA samples used.

Inhibitors of serotonin receptors were injected prior to L-DOPA to block the effects of serotonin released when dopamine is synthesized from L-DOPA. Methysergide produced no significant enhancement of spontaneous locomotor activity in male mice. Methysergide injected 20 minutes prior to L-DOPA had no significant effect on 560 or 1000 mg/kg of L-DOPA. Higher doses of methysergide reduced the depressant effect of the higher doses of L-DOPA. Chronic

administration had minimal and non-significant ability to reduce the depressant effects of doses administered just prior to the locomotor activity tests. No differences in dopamine and serotonin were observed after L-DOPA in animals which were chronically pretreated with L-DOPA or saline. However, norepinephrine levels were significantly lowered in the chronically pretreated animals. The mechanism of this depletion is being investigated.

L-DOPA prolongs the period of post-decapitation convulsions indicating an effect of L-DOPA on the spinal cord. This effect is not blocked by haloperidol, but is mimicked by para-chlorophenylalanine. It has tentatively been concluded that the effect is related to the depletion of serotonin produced by L-DOPA. The results demonstrate that L-DOPA acts, in part, by depressing the level of serotonin in the brain and also by producing release of serotonin on to serotonin receptors. The increase in post-decapitation convulsions may implicate a dopamine-serotonin interaction at the level of the spinal cord which influences spontaneous motor activity. As observed previously, numerous side effects have been encountered after the therapeutic use of L-DOPA. These results suggest that these side effects may result from the action of L-DOPA alone on the serotonergic system and that they may not require that L-DOPA be converted to dopamine in excess amounts or that dopamine must be formed prior to effects being observed in serotonergic systems of the brain.

The large patient population of Parkinsonism and allied diseases accumulated over the past several years continues to be documented and studied with emphasis on the following: defining the effects of pharmacological agents on the natural history of the disease; testing the therapeutic efficiency of newly developed agents; monitoring of side effects and toxic reactions of presently employed treatment programs; and searching for new insights as to the etiology and pathogenesis of these disorders. Central to this program are the developments regarding the effects of monamines, particularly dopamine, on the symptoms of basal ganglia disorders. These investigations are directed towards the application of these new findings from a diagnostic and therapeutic viewpoint as well as their implications as to the causation of these disorders. Essentially this program is continuing documentation of cases of Parkinson's disease and other types of Parkinsonism. In accord with a specially designed protocol, including a rating scale for Parkinson signs and symptoms, patients are evaluated at regular intervals. These data have been useful in delineating the natural evolution of the Parkinson process and determining the effects of various treatment programs. This patient population also acts as a resource for exploring biochemical abnormalities in CSF and other body fluids which may exist in this disorder. In this regard, emphasis at present is on the various monamines, their metabolites and enzymes as well as the essential amino acids. Additionally, autopsy material is periodically being derived and correlative clinical and pathological studies carried out. The long-term assessment of the therapeutic effectiveness of levodopa in Parkinsonism is being investigated. The large patient population under treatment will continue to be examined at regular intervals as to the state of their Parkinsonism, evidence of side reactions and/or toxic effects from the use of this agent. Here again, specific studies to identify metabolites of levodopa in CSF are in progress with a view to determining correlations with beneficial as well as side effects. When available, autopsy material is obtained and studied to determine the effects of administration of levodopa on the pathological Parkinson process in brain as well as its effects on other organs.

In general the therapeutic effectiveness of L-DOPA has continued to be superior to that of other therapeutic measures. It is, however, becoming increasingly apparent that it does not halt the progression of the disease and that after long-term usage, less uniform therapeutic response occurs and a greater incidence of CNS side effects develop. The two major side reactions limiting the clinical usefulness of levodopa are induced abnormal involuntary movements and a diurnal variation in therapeutic effects, so called "on-off" response. Special studies as to the incidence, nature and measures for control of these reactions are being carried out.

The studies so far indicate that 70-80% of patients on long-term L-DOPA therapy develop abnormal involuntary movements in varying degrees. They tend to make their appearance in that segment of the body first involved in the Parkinson process and are dose and time dependent. In patients coming to autopsy in whom such movements had existed, no morphological changes were found. Except for reduction of the daily dose of levodopa, no effective means for their control have been found. Over the past years clinical trials have been conducted with the following: L-tryptophan - administered in doses of 2-4 gms a day has failed to alter the movements, similar negative results have been obtained with 5HTP. A trial of N-Butyl-gallate-alpha-methyl transferase inhibitor did not alter these adventitious movements nor were they effectively diminished by the use of 3-O-methyl dopa. In an attempt to find clues as to the possible mechanism of these movements, extensive studies were carried out of the amino acid content of CSF in patients exhibiting this phenomena. In preliminary studies it was found that the free amino acid content of basal ganglia disorders is elevated. This is particularly true for leucine, valine and arginine. After prolonged L-DOPA administration and with the appearance of abnormal movements, these abnormalities are increased. The significance and implications of these abnormalities are being further explored.

"On-off" effect reaction which appears to be a complex form of a akinetic-rigid state, in its "off period" is intimately related to the use of L-DOPA. It bears a relationship to the time intervals of treatment in that in most instances it has made its appearance after months of treatment with L-DOPA. It occurs in some 15-20% of patients and its causation is unknown. Two patients were monitored for blood levels of L-DOPA over periods of 8-10 hours and marked alterations which can be correlated with the "on" and "off" periods were found. Both patients were unable to sustain a consistent uniform level of L-DOPA and the off periods occurred when their blood levels of L-DOPA dropped below a critical level of 5 $\mu\text{m}/\text{l}$. Attempts to sustain a consistent blood level alone, by increasing the daily dosage or shortening the dosage schedule resulted in less frequent "off" periods but an intolerable degree of involuntary movements. Alternate means of controlling this phenomena by lowering daily protein intake and using other anti-parkinson agents has to date been less than satisfactory. Further studies of this perplexing phenomena are contemplated.

A number of clinical trials involving new anti-parkinson agents are being carried out now.

N(5-pyrrolidine-3-pentynyl) succinimide is an analogue of oxotremorine which effectively blocks pharmacological action in laboratory animals. In preliminary trials carried out in 6 patients, a rapid effect in reducing tremor akinesia and rigidity in parkinson patients was observed. However, a number of side effects including dizziness, nausea and behavioral disturbances occurred. These latter may be related to dosages administered in these preliminary trials. A trial beginning with a more limited dosage schedule and an attempt to use this agent with L-DOPA will be carried out in the future.

DL Serine N-(2-3-4-trihydroxybenzyl)-Hydrazine Hydrochloride is a DOPA decarboxylase inhibitor whose action occurs primarily in extra-cerebral structures. When given in combination with L-DOPA it can substantially reduce the required dosage and reduce the incidence of side effects originating in peripheral structures. It has been administered to 6 patients who had previously received L-DOPA in maximal dosage and had less than an optimal response, and were experiencing side effects. In all patients it was possible to effectively reduce the required dose of L-DOPA by 70-80%. A therapeutic ratio of L-DOPA to the inhibitor of 4 parts to 1 was found most efficacious. However, the combination failed to fully obviate central side effects which some of these patients were experiencing. More intensive study of this agent is needed to fully establish its value in the treatment of parkinsonism.

Alpha-Methyl-Dopa-Hydrazine is also capable of peripheral DOPA decarboxylase inhibition. Trials to determine its therapeutic value in parkinsonism to date have been carried out on 184 patients. Alpha-methyl-DOPA-hydrazine effectively reduces the required daily dosage of DOPA, shortens the induction phase for DOPA, reduced the incidence of side effects such as nausea, vomiting and anorexia. To date, no side effects attributable to this hydrazine compound have been encountered. The therapeutic response is obtainable in most patients with a ratio of 10 parts of L-DOPA to 1 part of alpha-methyl-hydrazine. On the average, patients require 1.0 gm of L-DOPA and 100 mg of alpha-methyl-hydrazine which can be divided into 4 equal doses..

Following the initial report that L-DOPA was primarily methylated to 3-O-methyl-dopa compound which was then capable of being converted back to DOPA and dopamine, trial of its effectiveness in parkinsonism was initiated. In 8 patients treated with 3-O-methyl-dopa, up to 6 gms a day, no alteration in parkinson symptoms were obtained. Additionally, CSF studies failed to show a rise in DOPA following its administration though 3-O-methyl dopa rose to considerable levels. It is, therefore, been concluded that this agent is ineffective in the treatment of parkinsonism.

The search for a viral agent in Parkinson's disease, especially the post-encephalitic form is receiving increased attention. Recent observations demonstrated influenza virus antigen in brain sections of 6 patients with post-encephalitic Parkinsonism by direct immunofluorescence staining. Specific immunofluorescence was seen in nuclei of cells in the substantia nigra and hypothalamus, and was apparently strain-specific for the neurotropic strains of type A influenza virus. Such fluorescence was not seen in brain sections of 6 patients with idiopathic Parkinson's disease. These findings constitute the first important laboratory evidence, so far, that certain strains of influenza A virus may be linked with post-encephalitis Parkinson's,

and that the latter is probably etiologically different from idiopathic Parkinson's disease. This study, however, does not furnish absolute identification of the viral agent, nor does it presume to offer conclusive evidence for causal relationship. Moreover, these findings are difficult to fit with presently known clinical and epidemiological data, the postulated relationship of encephalitis lethargica, post-encephalitic Parkinson's and influenza having become less tenable than it had been in the 1920's. The brains of a well-documented case of post-encephalitic Parkinson's and a case of Parkinson's disease with dementia are now being studied.

There are two major routes of DOPA metabolism. The first involves decarboxylation to dopamine and subsequent catabolism of dopamine to O-methylated deaminated, and beta-hydroxylated products. The second major route of catabolism involves transamination processes to pyruvic and lactic acid derivatives.

It has been suggested that the prolonged time course of the therapeutic effect of L-DOPA results from the accumulation of one of its metabolites, 3-O-methyl dopa, which is slowly de-O-methylated to DOPA and subsequently decarboxylated to dopamine. A correlation between neurologic status, psychiatric status, and metabolism of L-DOPA is being sought. A population of patients with Parkinsonism is placed on a standardized regimen of therapy with L-DOPA. These patients are evaluated in great detail with regard to their neurologic status, psychiatric status and levels of urinary dopamine and dopamine metabolites in the drug-free state. The major finding thus far in this study of patients with primary Parkinsonism is that the pretreatment levels of each of the two metabolites of dopamine, dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA), are specifically correlated with the severity of the neurologic disease or with a specific indication of mental status. DOPAC varies with the severity of the neurologic disease, and HVA, with the psychiatric status. The primary objective of this study is to confirm these observations and to extend the findings to patients other than those with primary Parkinsonism. Additional correlations will be examined between the metabolites and the therapeutic as well as the adverse effects of the drug in an attempt to clarify the numerous problems and to lend additional data to evaluate the several theories which have been put forth to explain the clinical observations.

A strong relationship between the sulfate conjugation of a major metabolite of L-DOPA (3,4-dihydroxyphenylacetic acid, DOPAC) and the severity of Parkinsonism is suggested. It has been found that administration of large amounts of L-DOPA to patients results in saturation of the mechanism for sulfate conjugation of L-DOPA metabolites. It was, therefore, of considerable interest to explore the biochemical determinants of the sulfurylation process. The experimental approach used here is a comparative study of the sulfate-conjugating systems in the liver and brain which may participate in the metabolism of L-DOPA or its metabolites in the intact mammal. The program is directed to determine the relative capacities of the liver and brain to generate sulfate esters of L-DOPA and its metabolites, and to examine the ability of DOPA metabolites to saturate these systems, also to compare the enzyme systems of the brain and liver which generate the sulfate conjugates of L-DOPA and/or its metabolites with those reported to catalyze the production of sulfate derivatives of other substances (e.g., phenol, serotonin, estrone).

Small concentric hyaline spherules are a distinctive feature of the form of Parkinsonism known as Paralysis Agitans. These round structures, known as Lewy bodies, occur in various pigmented neurons throughout the brain stem (e.g., substantia nigra and locus coeruleus). They occur most frequently in idiopathic Paralysis Agitans, less frequently in the post-encephalitis variety, and small numbers of them are also encountered in 5-20% of "control" autopsies. When examined by electron microscopy, the bodies are composed of filamentous structures which are more densely packed in the central portion of the spherule. The precise biochemical nature of Lewy bodies is not known. Efforts are being made to collect these bodies in greater number for chemical and morphological analysis. The answer would be of interest in relationship to the problem of parkinsonism, even though the specific nature of the relationship is not evident at the present time.

Aberrant L-DOPA metabolisms: Norlaudanosolinecarboxylic acid (NLCA) and related tetrahydroisoquinoline alkaloids (TIQA) have been implicated as intermediates in Papaver alkaloid biosynthesis in higher plants and their formation from dopamine (DA) and 3,4-dihydroxyphenylpyruvate (DHPPA) occurs so readily under physiological conditions that there is a high probability of their formation in mammalian tissue under conditions of aberrant L-DOPA metabolism. Thus, the studies are directed toward showing the distribution of the TIQA in mammals, their role in L-DOPA chemotherapy and their effects on enzymes of catecholamine metabolism.

Techniques have been developed to derivatize these compounds for GLC, utilizing pentafluoropropionic anhydride or heptafluorobutyric anhydride to acylate phenolic hydroxyls and the amine nitrogens, and fluoropropanols for esterification of the carboxylic acid groups. In this manner volatile derivatives were obtained which have been separated and detected at picogram levels by mass fragmentography. Using these procedures, small amounts of NLCA have been found in the urine of normal individuals whereas 2-4x as much has been found in the urine of a Parkinsonian patient and in rats on L-DOPA chemotherapy. Furthermore, in preliminary findings NLCA has also been detected in brain tissue of rats on DOPA and carbidopa. Dopamine levels were also determined as a control and these were in good agreement with values reported in the literature for this L-DOPA regimen. Presently, levels of the other TIQA and DHPPA are being determined in brain, liver, kidney, gut and urine. Since the conjugated form of NLCA has been found in urine, gut, liver and kidney extracts will be subjected to glucuronidase treatment before fractionation and derivatization of the catecholamines. The condensation of dopamine and DHPPA to form NLCA occurs spontaneously in the presence of mammalian tissue such as brain, liver and kidney. Under physiological conditions yields of 1-2% have been observed and the identity of NLCA product has been confirmed by mass fragmentography.

Metal chelates of L-DOPA: In an effort to develop better drugs for the treatment of dopamine-deficient diseases, an extensive study concerned with the metal chelates of L-DOPA is being undertaken. The physical and chemical characteristics of these chelates will be studied, as will their transport into the brain of experimental animals. The interaction with pyridoxal phosphate and with aromatic acid decarboxylase will also be studied. The comparative peripheral versus central metabolism of these chelates will be

investigated in an effort to understand the mechanism whereby they are more efficiently transported into the brain than the unchelated L-DOPA.

The results of the exploratory studies have provided significant preliminary support to substantiate the validity and usefulness of the theoretical speculations that the pyridoxal-dependent decarboxylation of L-DOPA in the precerebral areas might be obviated by an appropriate metal chelation of the aminocarboxylate end of the L-DOPA molecule. In view of the very promising nature of the results, i.e., 100-150% increased transport of the amino acid into the brain with practically no associated metal levels in the brain, it is felt that, from the standpoint of potential significance, a detailed program of research on this topic would be very worthwhile and profitable. Further, through the exploration of a wide range of metal-L-DOPA chelates, the proposed research is directed toward not merely the delineation of their basic mechanisms but also presents the possibility of developing the chelated systems for the therapeutic application in dopamine deficient diseases. It is evident that the novelty of the metal chelation approach and the multi-disciplinary character of the research effort should prove to be very valuable toward achieving the desired objectives.

EPILEPSY

Although epilepsy has been known for centuries, it is still somewhat difficult to define the disorder in terms which will cover its manifold features. Yet it does seem obvious that epilepsy is not one clearly defined disease but rather is a disorder which is characterized by a persistent liability to recurrent, paroxysmal (sudden, episodic) attacks. In the majority of these attacks there is impairment of thought and awareness or responsiveness, or both, and there may or may not be convulsive movements or automatisms. In attacks in which the disorder remains localized, consciousness may not be impaired. The seizures may be associated with structural disorders of the brain, or with a toxic cause. More frequently, however, there is no discoverable organic disease of the brain with which seizures are associated. Some forms of epilepsy may be genetically determined.

A survey of the world's literature on the epidemiology of epilepsy, which involved many racial groups, revealed that at present a minimal prevalence of convulsive disorders is about 4-6 per 1,000 population. A prevalence rate in the range of 14.1/1000 to 20.1/1000 population was determined in another study in which a general survey of children was conducted. Clearly, epilepsy is a major national problem, regardless of the specific statistics used as indicators.

The clinical manifestations of seizures are so varied that an entirely satisfactory classification is difficult to make. In general, there are two ways of classifying patients with convulsive seizures. The first method classifies patients into two groups, symptomatic and idiopathic, according to the presence of known organic factors which may be of importance in the occurrence of attacks.

The second method of dividing patients with seizures is that of separating them into several groups according to the manifestations which occur during the attack:

Absence Seizures (Petit Mal)

The absence seizure is a sudden brief loss of awareness associated with electroencephalographic evidence of generalized spike and wave discharge. Clinically, the unawareness is manifested by a blank stare and unresponsiveness to ordinary conversational speech. The patient is usually totally unaware of surroundings or events during the brief seizure, but the older patient is aware of its occurrence because of an interruption in the stream of consciousness. These seizures have no aura. Both the loss of awareness and the return of full awareness occur abruptly. The majority of seizures rarely last more than 10 seconds. Mild clonic movements of the eyes or hands, head nodding, or automatic movements, alone or in combination, accompany most staring attacks. Absence seizures are usually inherited, and other members of the family may have clinical or subclinical absence attacks.

Partial Seizures with Complex Symptomatology (Psychomotor; Temporal Lobe)

These attacks are generally accompanied by impairment of consciousness but memory may be retained for bits or islands of conscious experience. Patients may recall peculiar thoughts, dreamy experiences, and peculiar sensations, or experience distortions of sensory perception. They may carry on automatic behavior which they cannot recall. The majority of patients suffering from partial seizures with complex symptomatology will experience an aura, and full consciousness returns gradually over a period of seconds or minutes. The attacks usually last for several minutes.

Partial Seizures with Elementary Symptomatology (Focal Motor Seizures; Jacksonian Seizures)

These attacks may include special sensory or somatosensory symptoms, autonomic symptoms or compound forms. Patients are usually able to describe this type of seizure quite accurately, and in adults a lesion of the brain associated with the focus is usually definable.

Generalized Tonic-Clonic Seizures (Grand Mal; Major Motor Seizures)

The generalized tonic-clinic seizure, although frequently occurring without apparent sequelae, may result in permanent brain damage or death.

Recurrent Generalized Tonic Clinic Seizures (Grand Mal; Major Motor Seizures)

Approximately half the patients with these types of seizures will have evidence of a lesion or a disorder of the brain as a cause for the seizures, even though there may be no history of aura or other types of seizures.

Status Epilepticus (Grand Mal Status Epilepticus)

Status epilepticus is the recurrence of one generalized tonic-clinic seizure after another, without full recovery of function between seizures. Status epilepticus is an acute medical emergency.

Currently the National Institute of Neurological and Communicative Disorders and Stroke is providing grant support for five Epilepsy Research Centers. They are located in New Haven, Connecticut; Houston, Texas; Seattle, Washington; Palo Alto, and Los Angeles, California. Research highlights of three centers are presented below.

Yale University, New Haven, Connecticut

The Neurology Research Center (Epilepsy) at Yale is organized as a widely based investigative effort into mechanisms of epileptic seizures and their control, and of phenomena associated with seizure states, with the aim of discerning factors responsible for the occurrence, frequency and nature of seizure disorders.

One investigator has been looking at the physiology of convulsant and anti-convulsant drug actions on identified invertebrate neurons. He has noted the

increased potassium conductance with drugs like phenobarbital. Data thus far from investigations of leech sensory neurons indicate that their action potentials depolarize faster than those of the Retzius cell, are more sensitive to tetrodotoxin, less sensitive to tetraethylammonium chloride and respond differently to barbiturates.

Another study, on the mechanism of action of diphenylhydantoin (DPH) has shown a marked increase in the spontaneous release of neuro-transmitter which depends on extracellular calcium concentration, and reflected in MEPP frequencies affected in a neuromuscular junctional preparation when this drug⁴⁵ is introduced. DPH also was found to reduce brain mitochondrial uptake of Ca⁴⁵.

Parallel investigations with ethosuximide also have been started. Effects of various convulsant and anticonvulsant drugs on cerebral evoked potentials in the cat are also being performed. Studies with anticonvulsant drugs are ongoing; Phenobarbital in moderate dosage (to blood levels of 16 µg/ml) has not affected SEPs recorded from cortical and subcortical regions. DPH, to blood levels of 10-20 µgm/ml, produced SEP changes mainly in subcortical structures and cerebellum, not cortical (S1). SEPs from VPL, centrum medianum and mesencephalic reticular formation showed a prolongation in latency and suppression of a secondary positive component.

Convulsive seizures in animals induced by allylglycine, pentylenetetrazol and flurothyl were found to produce increases in cerebral gangliosides and decreases in cerebellar ganglioside sialic acid levels.

Clinical investigations are an important component of this Center. About 230 patients are being evaluated in special EEG-TV split screen monitoring units. Special attention has been paid to the relationship between alcohol intake and epileptic seizure activity. Twenty percent of non-alcoholic epileptics reported seizure frequency increased by alcohol usage; this was particularly related to actual amount of alcohol intake and the "withdrawal" or "morning-after" seizure occurrence was emphasized. Actual alcohol administration, in controlled investigation, reinforced the pattern of delayed or withdrawal effect. A reduced seizure discharge effect of alcohol on EEG spiking was demonstrated. Alcohol also was found to suppress photically induced myoclonus or convulsive activity, especially in the EEG, during the period of alcohol administration. Further investigations are attempting to relate these effects more specifically to seizure type and particular EEG patterns.

A study of compliance in anticonvulsant drug therapy includes 154 patients. Compliance appears to be better in patients receiving a complex medication regimen; however this may merely mean that such patients who actually are more consistent and cooperative are given such a regimen. Non-compliers have been found to react more depressively in psychological testing. The complex data collection and analysis in this group is continuing to attempt to establish group norms for each category of variables.

The use of dimethoxymethyl phenobarbital (DMMP) therapy in epilepsy is being evaluated. The DMMP study has involved now 53 patients, 27 in a double blind study, as well as some in acute toxicity evaluations. A method for the determination of the metabolite, MMMP, has been developed. In a cross-over

study with phenobarbital, the overall effect on seizure frequency was statistically similar; yet, in some patients the decrease in seizures was associated with less side-effects. This is being evaluated further in the long-term study group.

Studies of cerebral sensory evoked potentials (SEP) in epileptic patients are continuing. Until recently, investigations of SEP from scalp recordings in epileptic patients revealed no consistent specific abnormalities or changes correlated with type and severity of seizure, or localization of discharge, apart from those found in myoclonic seizure states. During this past year, a SEP correlate of an epileptogenic focus has been found: It is observed as a large negativity, from both scalp and depth recordings in some patients with a temporal lobe epileptogenic focus, designated as the "epileptic wave" or "E" wave. It is not actually generated from the temporal lobe focus itself; it appears to generate from post-central gyrus, being given altered form by the focal epileptogenic activity.

Feedback modification of EEG activity and its possible role in reduction of epileptic seizures is being done in normal and epileptic patients. Thus far in 5 epileptic patients in a research protocol involving feedback training in a single-blind crossover design, followed by a second crossover to random feedback, then contingent training, significant seizure frequency reduction resulted in three patients. This occurred particularly after the second contingent training, and was not correlated with any changes in 12-14 Hz activity in the EEG, even though 9-14 Hz feedback was used. Yet, alpha rhythm was enhanced consistently.

University of Washington, Seattle, Washington

At this Epilepsy Center an emphasis is on animal models in which convulsions are induced, followed by physiological and morphological studies of brain tissue. One such study attempts to document the degree of spatial and temporal synchrony of neural activity in advanced alumina foci. Since such synchronous neural activity represents continuous "kindling" of the focus, its source and modifiability are of considerable experimental interest.

Some data suggest that electrophysiological activity does not begin at one cortical point and spread to adjacent areas; potentials usually appear simultaneously over the whole focus. An interesting highlight is that when a monkey's seizures are continuously monitored throughout the study, after several months of recording, the seizure frequency dropped to zero suggesting that variables involved in recording, possibly electrode penetration, may effectively reduce the epileptogenic potency of the focus.

Single unit recording on the cervical spinal cord of awake monkeys is also being done. Investigators have recorded the cells in cervical spinal cord of several monkeys trained to make alternating wrist movements. Single units in dorsal and ventral horn were characterized with respect to responses to natural stimulation of the arm responses to electrical stimulation of brachial plexus nerves. Besides confirming that long-term recording of spinal cord cells in awake monkeys is feasible, this study has shown that many dorsal cord cells responsive to cutaneous stimulation are inactive in the absence of stimulation.

Cortical glia developing in rhesus monkeys with experimental epilepsy is under study. Subarachnoid alumina resulted in epilepsy in 4-6 months and the lesions spread over the cortex like an umbrella. An astrocytic reaction in superficial cortex was general, but in deeper layers organized stripes or bands of giant hypertrophied astrocytes were seen at depths where potassium electrode studies have shown increased potassium ions in extracellular spaces with seizures. Astrocytes are thought to contribute to or cause epileptic lesions. They appear to respond to the resulting hyperactivity in neurons, associated with ion flux or transmitter release, by hypertrophic reaction in the epileptic focus.

The movement of the tracer horseradish peroxidase (HRP) in the cortical intercellular space (ICS) in both normal and epileptic monkey cortex is being studied. Data indicate that the nature of the extracellular space adjacent to the epileptic lesions in epileptic cortex has been modified. Penetration of HRP was more rapid into the cortical alumina granuloma than any other site, but HRP was impeded from movement out of the granuloma into the surrounding brain. Abutting the lesions, HRP moves through the ICS in gliotic cortex more slowly than elsewhere. Either there is impeded movement through scarred epileptic cortex, or diminished intercellular space or both. The micro-environment of neurons and their processes being thus affected may be the greatest factor contributing to epilepsy.

Research continues on anticonvulsant pharmacokinetics in monkey. The pharmacokinetic profile of Ethosuximide (ETHO) was studied in a group of six chronically catheterized male rhesus monkeys at three dose levels intravenously and orally. Plasma and urine levels were assayed by GLC. In order to establish chronic dosing regimens of ETHO for efficacy testing, two types of infusion experiments were implemented in a group of six chronically catheterized male rhesus monkeys. In both studies, the difference between theoretical and experimental mean plateau plasma concentrations was not statistically significant. Also, total body clearances, volumes of distribution and elimination half-lives determined from least squares fit of the infusion data were not statistically significantly different from the respective parameters obtained from single-dose studies.

The relationship between infusion rate and blood levels has been investigated during and after constant-rate intravenous infusion of Carbamazepine for 8 to 12 hours in six rhesus monkeys. Examination of serum concentration curves indicated a more than proportional increase in steady-state concentrations with an increase in infusion rate in monkeys. It was concluded that Carbamazepine exhibits dose-dependent kinetics following short-term infusion in monkeys and that a model with zero-order input and one capacity-limited elimination pathway is adequate to describe the pharmacokinetic behavior of the drug.

The cerebellum is being studied as a site for extrafocal action of diphenylhydantoin (DPH). DPH is known to prevent the development of propagation of cortical epileptiform activity. This effect is apparently not exerted through direct depression of epileptogenic tissue, but is thought to be exerted partially through stabilization of non-epileptic neurons and partially through reinforcement of extrafocal inhibitory systems within brain which serve to

limit seizure propagation. It was observed that surgical extirpation of cerebellum resulted in augmented epileptiform after discharge responses of suprasylvian gyrus as a consequence of direct repetitive stimulation of cortical surface. In addition, there is evidence suggesting that DPH may exert direct effects on the cerebellum.

Cerebellar ataxia, both reversible and irreversible has been described following chronic administration of large doses of DPH; selective Purkinje cell degeneration has also been demonstrated in cat cerebellum, and correlated with DPH blood levels in human, cat, and rat.

The effects of antiepileptic drugs on cerebellar Purkinje cell discharge rates and cortical epileptiform burst (CEB) activity were studied in cats with penicillin foci in sensorimotor cortex. In controlled experiments, extracellular microelectrode recordings of Purkinje cell activity revealed characteristic low frequency discharge rates during periods of cortical quiescence and discharge rates of 150 Hz or more occurring concomitantly with focal cortical spike activity. Cell discharges abruptly ceased during development of CEB's which became generalized and maximal in both cerebral hemispheres. Following DPH, CEB activity and duration was markedly reduced and sustained Purkinje cell discharge rates of 140 Hz were recorded. Following cerebellectomy in DPH-treated animals CEB activity intensified, was more frequent, involved both cerebral hemispheres and was of much longer duration.

These investigators have also demonstrated that acutely isolated and normal intact cerebral cortex of cats displays an increase after discharge duration after 10 minutes of ethosuximide administration doses of 100-400 mg/kg I.V. In isolated cortex, this increase is enhanced by atropine pretreatment and abolished by physostigmine pretreatment. In intact cortex this increase was reduced by atropine pretreatment. Thus, intact and isolated cortex react differently to cholinergic agonists and antagonists.

It is widely accepted that acetylcholine acts as an excitatory transmitter in the central nervous system. However, the effect of cholinergic agents on the isolated slab may indicate that subcortical connections play an important role in the response of intact cerebral cortex to these and other drugs. Data suggest that the transient increase in epileptiform after discharge duration after ethosuximide administration is due in part to some cholinergic mechanism and may reflect the depression of cortical inhibitory pathways. Different responses of intact and acutely isolated cortex indicate the importance of consideration of subcortical influences in any study relating to drug effects on cortical functions.

The effect of ETHO, DPH, trimethadione, and phenobarbital on the conjugated estrogen induced epileptiform activity was studied to aid in determining the utility of this model in screening potentially useful petit mal antagonists. The effects of DPH were determined on time interval histogram generated by conjugated estrogen application to the cortex. 2.5 mg/kg of DPH augmented the number of intervals shifted the peak of distribution to 60-70 msec. Higher doses of DPH 5-20 mg/kg caused a progressive reduction in intervals without changing the distribution peak.

ETHO-produced dose related changes in the time interval histogram. Fewer intervals were recorded after each dose and by 600 mg/kg few interspike intervals were seen. The effectiveness of phenobarbital of focal epileptiform activity generated and by conjugated estrogen topically applied to the cerebral cortex was demonstrated. Application of conjugated estrogen to the cortex by means of chronically indwelling drug-matrix-recording electrodes has been carried out in order to ascertain whether or not behavioral correlates of the 3/second spike interval wave activity existed. However, over a 4 or 5 day period in which conjugated estrogens leaked from the plastic matrix, animals exhibited myoclonic jerking and occasional seizures while demonstrating either 3/second spike activity or polyspike trains in the case of major seizures. This is the first time to our knowledge that any behavioral work has been done with conjugated estrogen epilepsy.

The efficacy of pharmacologic prophylactic treatment of post-traumatic epilepsy was explored in the monkey model. Administration of DPH and phenobarbital in a combined regimen commenced within 48 hours of alumina gel injections. After one year the monkeys were withdrawn from either their drugs or placebo and followed for a subsequent four-month period. The data indicate that anticonvulsant treatment of potentially epileptic monkeys (a) decreases both the frequency and severity of seizures they would have had without treatment, and (b) early drug treatment is more effective than later drug treatment. A subsequent study was concerned with the permanent effects of the pharmacologic treatment of post-traumatic epilepsy.

One of the more difficult problems to assess in epilepsy has been the influence of social phenomena on seizure occurrence. Although there is considerable clinical evidence that social situations may effect the amount of seizure activity epileptics manifest, systematic and controlled quantification of such interactions are rare.

Initial data on dominant-submissive behaviors, mother-infant interactions, and peer relationships strongly suggest that seizure occurrence is influenced by the social environment. Dominant epileptic monkeys are able to maintain group control if their seizures are not too frequent. Submissive epileptic monkeys have seizures when threat-chased by dominant monkeys. In contrast to dominant epileptic monkey mothers, submissive mothers are incapacitated by seizures and unable to prevent the stealing and abuse of their infants by other monkeys. Reared-together peer monkeys protect and comfort the other when seizing.

In the treatment of focal motor and secondarily generalized epilepsy, DPH and phenobarbital are often prescribed together. One study involved the drug interactions in terms of plasma levels during ten-day periods of multiple dosing in four monkeys. The plasma drug-level data indicate self-induction of DPH, DPH and phenobarbital absorption, and systemic interactions and a decrease in the biological half-life of DPH when administered with phenobarbital. Additional data suggest a relationship between sleep stages and plasma drug level.

A major research activity for the Center Seizure Clinic has been the evaluation of new anticonvulsants by means of an extensive protocol. Data suggest that

Tegretol is a highly effective primary anticonvulsant able to deliver improved seizure control with fewer side effects than DPH when each is used separately.

Neuropsychological measures of cortical and subcortical function have been obtained. Two findings are notable. One is confirmation of the ability of thalamic stimulation at the time of input of verbal information to enhance later retrieval of that information some days later. The second has been the finding in one patient of an area undergoing cortical stimulation during the test of short-term verbal memory of an area in parietal operculum where stimulation during the distraction period regularly blocked retrieval from short-term memory, while stimulation during any other portion of the task was without effect. These findings suggest two things. First, there is a complete dissociation between memory and language functions in the cortex in contrast to the thalamus, the dominant hemisphere, and second, the parietal or perculum in the dominant hemisphere may be concerned with storage functions in short-term memory.

University of California, Los Angeles

This Epilepsy Center has focussed its efforts on an almost unique opportunity to develop useful methods to treat otherwise intractable cases of focal epilepsy while at the same time permitting studies of basic mechanisms in various types of cortical epilepsy, such as the electrophysiological changes, special studies of pathological substrates, neuropsychological alterations associated with these epilepsies and correlations with neuronal firing using microelectrode techniques. This has been made possible by the development of methods to stereotactically implant chronic macro- and microelectrodes in epileptogenic cortical areas previously inaccessible to conventional methods and to make long-term observations of such patients under all behavioral conditions including spontaneous seizures.

The group has recorded spontaneous seizures in patients using a 12-channel radio telemetry device. Contrasts are emerging between the characteristics of ictal disorders as seen in limbic system sites and in focal sensorimotor seizures. Trains of rhythmical seizure discharges can exist for 10 to 30 seconds within one or two sites in the hippocampal formation, which, if no further spread occurs, are not accompanied by any clinical seizure manifestation nor by any observable attentional defect.

Closer observations of the correlations between EEG changes and behavioral manifestations has been made possible by simultaneous filmed recordings of behavior and the EEG recordings with a split screen videotape method. The seizure detector made 46 correct detections, 20 of which went unreported by the nurse or patient. In most of the 20 the electrographic seizures were unilateral and probably had no significant behavioral counterpart.

In the past year seizures (both clinical and sub-clinical) were recorded with microelectrodes in patients. In most cases, these were sub-clinical or low grade attacks which were accompanied by distinctive EEG patterns which remained localized and little or no changes in unit activity were found. However, when focal seizure activity spread contralaterally and there were clinical signs, the focal unit discharge rate prior to the spread increased

in close relation to the high frequency and low amplitude phase of EEG seizure onset. During the clonic phase of the clinical seizure, firing was related to the sharp wave and not to the subsequent slow wave. Varying degrees of post-ictal depression of unit firing were found. Analysis of interictal firing patterns has revealed more instances of unilateral "bursting" which may be related to some structural neuropathology.

A computer method for EEG analysis has been developed for detecting the epileptogenic pacemaker of spontaneously occurring seizures and one which demonstrates that abnormal discharges spread through the brain with a time-course that can be followed. Studies comparing the effects of diazepam and phenobarbital on the spontaneous EEG of the limbic system and cortex have been carried out in 11 patients with intractable temporal lobe epilepsy undergoing depth electrode studies. While both drugs affected the cortex, diazepam had much more marked effects over a wider range of frequencies than did phenobarbital. Subcortically, only diazepam affected spontaneous activity. Diazepam rapidly suppressed spiking during the course of injection in non-epileptogenic sites but tended to transiently activate spiking in epileptogenic sites. Phenobarbital produced only mild spike suppression.

Observations on the structural substrates of seizure foci in the human temporal lobe have been made under light microscopy, electron microscopy and by Golgi methods. The most important finding disclosed in these studies is evidence of on-going degeneration in the neurons involved by hippocampal sclerosis.

There is increasing evidence from epidemiological studies that the 'partial epilepsies' and secondary (etiology known) generalized epilepsies represent a large fraction of the total problem of the epilepsies. Also the epilepsies are probably second only to CNS vascular disorders as the commonest neurological handicap. 'Partial epilepsies' are the most difficult-to-manage category including medical management and psychiatrically. Although surgical management in this group is possible only in a small fraction such methods appear worth advancing.

Turning to individual research grant activities, one investigator is analyzing presynaptic factors, causatively related and unique to epileptogenesis. Early pilot studies analyzing miniature postsynaptic potentials (MPSPs) in normal and epileptogenic cortex suggested that the impulse dependent MPSPs were diminished in epileptogenic cortex in distinction to the non-impulse related MPSPs.

Recordings were obtained from the lateral geniculate nucleus (LGN) ipsilateral to penicillin treated occipital cortex. Recordings of impulses from the LGN revealed impulses with prolonged IS-SD intervals, as well as failure of the SD spikes occurring coincidentally with the cortical penicillin wave. Such a prolongation of the IS-SD interval confirmed by failure of the SD spike is strongly suggestive of ectopic impulse generation. To confirm the fact that such impulses were antidromically invading the LGN soma, the geniculo-calcarine tract was stimulated simultaneously with the occurrence of an impulse at the LGN soma. When the antidromic impulse produced by the stimulation could be

recorded at the soma, this was evidence that the impulse "collision" did not occur and the impulse triggering the stimulus was also moving antidromically.

Recordings were obtained from LGN neurons which manifested the following action potential patterns during a cortical penicillin wave: (1) antidromic burst of impulses; (2) orthodromic burst of impulses; (3) a decreased impulse frequency; and, (4) no change in impulse frequency. The type of firing pattern appeared to be related to the relationship of the epileptogenic focus to the terminal axon of the LGN cell.

The ectopic impulse generation demonstrated to date has implications regarding epileptogenesis. If such impulse generation can be shown to precede or occur independently of the cortical penicillin wave and occur only in cells projecting to epileptogenic cortex, it may represent a previously undescribed mechanism of epileptogenesis. The burst of impulses could provide sufficient synaptic drive to produce the now well-known paroxysmal depolarization shift (PDS) in the postsynaptic cell characteristic of epileptogenic cortex. The additional feature of such a mechanism would be its relative independence from ongoing inhibitory synaptic activity.

One laboratory is continuing to develop and expand its capabilities for simultaneous monitoring of electrical and metabolic activity from the cat cerebral cortex and to apply these techniques to investigations of the mechanisms by which certain pharmaceutical agents affect CNS function. Recent data by means of reflection spectrophotometry have shown that this technique, together with microfluorometry offers the unique possibility of measuring noninvasively the oxidation-reduction level of intramitochondrial NADH and cytochromes at the oxygen end of the respiratory chain.

At another laboratory an investigator is examining the "kindling" phenomena involving different functional brain sites and different species in order to develop a reliable tool for assessment, exploration and recruitment of potential new antiepileptic agents, as well as to gain basic information regarding the development of pattern of epileptogenic brain processes. Initial work has been directed toward (1) gaining basic information as to the pattern of seizure development across species and mechanisms underlying the seizure development and (2) evaluation of known antiepileptic agents as well as cannabinoids with kindling and other "epileptic" animal preparations.

The basic phenomenology underlying amygdaloid seizure development in intact and split brain cats, has been described. A similar attempt has been completed in Senegalese baboon, *Papio papio*, which has natural antiepileptic seizures susceptibility. The chronological pattern of electroclinical seizure development suggested vertical intra-hemispheric ictal dissemination to be of primary importance for the progressive seizure development. Some animals developed spontaneous recurrence of both partial complex and primary generalized seizures. The basic information has been utilized for the assessment of antiepileptic agents.

At the University of California, Davis Campus, the breeding program designed to investigate onset and development of seizure response in young baboons and to provide information about heritability of the sensitivity to seizures has

continued. Data continues to be collected on the development of epileptic response to light with age. Thus far, the data do not yet allow firm conclusions regarding the heritability of seizures. Some suggestive results are beginning to emerge, however. One male with no history of seizures was mated with one female with mild seizures who has had three offspring, of which one is still non-epileptic at 18 months, one is usually non-epileptic but has had irregular positive responses since 16 months and the third one is asymptomatic at 7 months. Four offspring of a moderately stable, seizure-prone male and two different females show either mild unstable positive epileptic responses or are non-epileptic thus far. The most stable positive male has had most of the offspring surviving to be tested at 6 months:

- 3 stable epileptics from 1 epileptic female
- 4 stable epileptics from 1 epileptic female
- 1 stable and 1 mild epileptic from mildly epileptic female

Anticonvulsant compounds are being studied by the same group. Studies were completed on three analogues of amphetamine. Anticonvulsant activity, qualitative electroencephalographic changes and alterations in spectral density of spontaneous activity in various cortical areas have been correlated with doses of the three compounds. Fenfluramine, norfenfluramine, and 2-(1-m-tri fluoromethylphenyl)-2-propylamino-ethyl benzoate (S992) were administered.

Fenfluramine and norfenfluramine usually blocked seizure response to flashing light at doses of 1 to 5 mg/kg although some irregularity in response of one especially seizure-prone animal was noted. Norfenfluramine appeared slightly more potent when the two compounds were tested in the same animal. S992 produced almost no visible effects at doses up to 90 mg/kg. EKG, behavior, and EEG were quite similar to those of the control. At 40 mg/kg, two of four animals tested showed complete blockade of the seizure response to flashing light while two others appeared to have unusually severe seizures.

EEG changes induced by the fenfluramine and norfenfluramine were studied both visually and by analysis of sequential power spectra using a laboratory-based computer complex of LINC, PDP 12 and Time-Data 100. With fenfluramine, 1 mg/kg or more induced bursts of large, slow (1-5 Hz) waves over the cortex with lowest amplitudes over occipital and lateral frontal areas and highest amplitudes in parietal areas and moderate amplitudes in frontal and temporal cortex. Background fast activity was depressed. At higher doses, overall power in cortical waves was reduced. In contrast, norfenfluramine induced differing effects depending on dose. At 1 mg/kg, increases were noted in faster frequency (15-25 Hz) activity with reduced amplitude and a minimal increase in slow (1-5 Hz) waves. At higher doses, the EEG was dominated by 1-5 Hz slow waves with overriding fast activity and an overall increase in amplitude.

A group at Cornell University Medical College has been investigating metabolic changes in tissues exhibiting focal epileptic discharge and within areas of seizure spread. They have established models for focal seizures in the rat with the use of penicillin. They are able to induce centre-encephalic or cortico-reticular epilepsy with intravenous injections of penicillin. Assays were performed for ATP, phosphocreatine, glucose and lactate with the use of

the techniques of enzymatic cycling. Although there were no significant changes during the "interictal discharge," during the ictal discharge high energy phosphate compounds fall and tissue lactate rises in the focus. The metabolic rate is greatly accelerated within the focus and is capable of keeping up with the demands of the interictal discharge, but energy production falls behind energy consumption when the ictus begins. They also find that during seizures it is likely that glucose is metabolized primarily for the production of energy with end metabolites being lactate and CO₂ and not amino acids. Apparently glucose intake is significantly increased and it is shuttled to energy production at the expense of anabolic needs of amino acids and protein synthesis.

The final project reported here is concerned with the determination and comparison of molecular structures of chemically dissimilar CNS drugs which are clinically useful against similar pathologies, in order to discover stereochemical principles responsible for these efficacies. Progress has been achieved with the structural determinations of the anticonvulsant drugs ethyl phenacemide and phenacemide ("phenurone") and the dopaminergic agent Et 495 ("peridedil"). Preliminary structural results have also been achieved for the general anaesthetics ketamine and tiletamine.

Previous structural researches have conclusively demonstrated that diphenylhydantoin and diazepam, two of the most important clinical anticonvulsants, and procyclidine and trihexphenidyl, drugs with demonstrable laboratory efficacy against induced seizures of the grand mal type, share certain stereochemical features in their three-dimensional conformational structures. Experimental results have contributed evidence that (1) the stereochemical characteristics postulated to be responsible for the anticonvulsant action of the drugs studied are in fact the necessary features, and (2) diphenylhydantoin, diazepam, procyclidine, and triexphenidyl likely share the same mechanism of anticonvulsant action and act through the same receptor site.

MUSCULAR DYSTROPHIES

Current distinctions between neuropathies and myopathies are based upon distinctions made by the German-French fathers of contemporary neurology about 100 years ago. Although these distinctions are being challenged by such compromise terms as "neuromyopathy," there is still a defensible rationale to the traditional analysis.

Muscular dystrophy, in this presentation, refers to myopathy with two specific characteristics: a genetic basis and progressive weakness. The progressive muscular dystrophies are characterized by weakness and degeneration of the affected muscles which tend to follow a characteristic distribution in the members of the affected family. The exact cause of the degenerative changes in the muscles is not known. However, there appear to be various disturbances in the enzyme systems concerned with muscle metabolism.

Significant pathological findings tend to be confined to the muscles although there may be a few degenerative changes or a slight reduction in the number of ventral horn cells. In the early stages of the disease the muscle fibers are rounded and enlarged to more than twice their normal size. With progress in the disease, there is a longitudinal splitting of some of these large fibers with resulting admixture of fibers of various sizes.

There tends to be a general vacuolization and degeneration of the myoplasm with an increase in the number of sarcolemmal nuclei and replacement of the muscle substance by fat and connective tissues.

The progressive muscular dystrophies are currently divided into three categories which are justified primarily on the basis of prognosis. The categories are: Duchenne, Facioscapulohumeral, and Limb-girdle. The Duchenne Dystrophy is primarily a disease of childhood, whose predominant target is the male. It tends to begin at the pelvic girdle with a pronounced pseudo-hypertrophy. It progresses at a rapid rate with marked deformity and is considered to have a sex-linked recessive characteristic.

The facioscapulohumeral type is a disease of adolescence, attacking both males and females; seldom showing the pseudo-hypertrophy. It is initially localized at the shoulder girdle and always involves facial symptoms. It tends to progress rather slowly, with rare indications of deformity. Transmission is by an autosomal dominant gene.

The Limb-girdle type occurs generally between childhood and thirty years of age. It attacks both males and females during the first thirty years. The pseudo-hypertrophy is uncommon but involvement may include both the pelvic and shoulder girdles. The face never appears to be involved and the rate of progression is moderate. Only occasionally is there deformity and the genetic characteristic may be either recessive or dominant.

Myotonic muscular dystrophy is an inherited disease characterized by myotonia, weakness and wasting of the muscles especially those of the face and neck, cataracts, early baldness, testicular atrophy and evidences of dysfunction of other endocrine glands. The disease is inherited as an autosomal dominant

trait. However, there is currently no adequate explanation of the various forms of pathogenesis. The muscular wasting is similar to that which occurs in progressive muscular dystrophy and is probably related to a metabolic defect. The difficulty in relaxation is due to repetitive firing of muscle fibers. The repetitive polarization is independent of neural influences and as yet is not understood.

It is generally believed that there are approximately 10,000 cases in the United States at the current time.

Diagnosis is usually made with the onset of muscular weakness in childhood--and presence of muscular pseudo-hypertrophy--family history and increased serum enzyme activity. The electromyogram and biopsy of the muscles are also of value in establishing the diagnosis. Diagnosis of myotonic muscular dystrophy is usually made from the characteristic of wasting of the muscles of the face and neck and the myotonia of the extremity musculature. A family history of the disease, cataracts and evidences of glandular dysfunction are also present in the majority of cases.

There is considerable variation in the course of these diseases. Prognosis is most favorable when onset of symptoms occurs after the first decade of life. It is not uncommon to find patients who have suffered with the disease for 40 or 50 years still able to walk. In the Duchenne form, it is common for the disease to progress from 5 to 15 years to the stage where the patient is bedridden or restricted to a wheelchair. Death may occur from infection or involvement of the bulbar respiratory and cardiac musculature.

There is no treatment which has proven to be effective in arresting the course of the disease. Stretching of contractors, bracing and tendon-lengthening operations are used with varying degrees of enthusiasm.

Myotonia may be relieved by the administration of quinine or procaine amide is often used to alleviate the myotonia. Dilantin has been reported to be of value in the control of myotonia. There is no treatment for the muscular weakness or wasting.

The neuromuscular clinical research center at Duke University, Durham, North Carolina, supports a variety of activities related to the muscular dystrophies as well as myasthenia gravis. One project is concerned with the biochemical and biophysical nature of myotonic dystrophy. In this study the objective is to examine red blood cells and muscle membranes obtained from patients with myotonic dystrophy for biochemical and biophysical abnormalities. It is hoped that this study will reveal a generalized membrane defect in this disease and provide information concerning the membrane characteristics that produce myotonia and may play a role in the development of dystrophic changes in muscles. Protein phosphorylase activity is determined in erythrocyte ghosts and isolated muscle membrane from normal subjects and patients with myotonic dystrophy. The demonstration of a decrease in protein phosphorylase activity in membranes from both red cells and muscle of myotonic patients suggests that there may be a more generalized alteration in membranes in this disease than had been previously postulated and/or a hereditary defect in red

cells that had not been previously recognized. This study may also reveal whether or not reduced protein phosphorylase activity of red cells and muscle membranes is limited to patients with myotonic dystrophy; if so, the study will then have diagnostic value. If the degree of change has any correlation with severity of disease, the test might have prognostic value as well.

Another study involves the electronmicroscopic scanning of erythrocytes from myotonic patients to determine the existence of a membrane defect in the cells. Preliminary studies have disclosed cells with a characteristically abnormal shape (called "stomatocytes") that are more common in the blood of patients with myotonic dystrophy than in normals. The specificity and reproducibility of the cells are being determined. The investigator hopes to eventually utilize this procedure as a diagnostic test and to find asymptomatic carriers of the disease if such exist.

In yet another project in this program two primary research objectives have been advanced. In one the uptake of calcium by a sarcotubular preparation and the ATPase activity of a myofibrillar preparation is being measured in muscles that have different isometric contraction times. These measurements will constitute a test of the theory that contraction time is related to sarcotubular calcium intake rather than ATPase activity. The other research objective is to test a theory that basic p-nitrophenylphosphatase (p-NNPase) correlates with some undefined process that governs passive potassium permeability, resting membrane potential, and indirectly the chloride conductance and other factors involved in production of myotonia, dystrophy and other neuromuscular disorders.

The demonstration of the possible generalized membrane abnormality in myotonic dystrophy may be of significance and the importance of the finding, its specificity, causal relationship to the disease, and diagnostic utilities is to be ascertained. It is notable that the interface between clinical and basic scientists is particularly strong in the projects being done under the aegis of this program.

Circumstantial evidence obtained at a clinical research center for neuromuscular disease in New York City implicates the surface membrane as a possible site of the basic genetic fault in Duchenne dystrophy. Adenyl cyclase has been shown to be a sarcolemmal enzyme and has been studied in homogenates of human muscle. In normal muscle, the enzyme is stimulated by epinephrine and sodium fluoride. In Duchenne and facioscapulohumeral dystrophies, responses to these activating agents were much diminished in contrast to other muscle diseases or denervated muscle. Cells have been grown in culture and during the coming year it will be possible to determine whether this abnormality is expressed in vitro. If so, it will provide additional evidence of a genetic fault in the muscle membrane.

The polyamines (spermine, spermidine, putrescine) modulate RNA and protein synthesis. Investigators have found increased content of putrescine and spermidine in dystrophic mouse muscle. Metabolic studies are in progress to understand the basis for this abnormality and similar studies will be done in normal and dystrophic human muscle.

Another laboratory has established that diethylstilbestrol (DES) lowers the high serum enzymes characteristic of boys with Duchenne's muscular dystrophy (DMD). Investigators are attempting to determine by what mechanism DES lowers the serum enzymes in this disease. They have reported that DES reduced the enzyme efflux from mouse skeletal muscle. Dose-response characteristics have been obtained over a dose range of 20 - 500 $\mu\text{g/day/mouse}$. The maximum reduction was 50% at the 100 μg dose, and larger doses were not more effective. Creatine phosphokinase (CPK) and lactate dehydrogenase (LDH) efflux could not be completely suppressed by this agent alone. The effects of prednisolone were also studied. Increasing doses up to 35 μg of prednisolone sodium-succinate every other day reduced enzyme efflux a maximum of 30%. Higher doses increased enzyme efflux. The effects of combining DES and prednisolone are now under study. The increased enzyme efflux associated with certain prednisolone doses does not occur when DES and prednisolone are given at the same time.

Studies of serum enzyme changes in 12 children with Duchenne's muscular dystrophy who received diethylstilbestrol for one year or more have been completed. These showed that the mean activities of the two serum enzymes CPK and LDH, which presumably derive from skeletal muscle, fall about 40%. Another enzyme (alkaline phosphatase) arising from bone increased. In addition, eight boys with DMD who are receiving prednisolone, alone or in combination with DES are under study. These will be completed in the near future, but they already indicate that: a) prednisolone also lowers the high serum enzyme activities in this disease; b) it is about as effective as DES in this regard; and c) when the two agents are administered together, the effects of these two hormones are additive. The effects of these agents on several metabolic, growth, and muscle strength parameters are continually being assessed, to evaluate both their safety and efficacy. Both agents appear to produce salutary effects in this disease.

Presumably, the high serum enzyme activities in dystrophy reflect enzyme leakage from diseased muscle. DES is the first agent to be shown to consistently lower the serum enzymes in DMD. This fact and more recent observations regarding the effect of DES on enzyme efflux from mouse skeletal muscle, have provided a rational approach to the evaluation of potentially therapeutic agents. Recently, a number of investigators have begun to use the serum enzyme in dystrophic patients; it has been found that the high serum enzyme activities are lowered by lithium and prednisolone (in man), and penicillamine (in the dystrophic chicken). It is important to establish that these agents act by reducing the enzyme leakage from diseased muscle, without reducing the skeletal muscle enzyme content. The mouse assay permits evaluation of the effect of a potential therapeutic agent on muscle enzyme content and the efflux of enzymes from isolated skeletal muscle, but in each instance direct proof of similar effects in dystrophic man will also be required.

Another investigator is attempting to determine if inherited human muscular dystrophies may be caused by defects in ribosomal protein synthesis. Protein synthesis in facioscapulohumeral muscular dystrophy (FSH MD) differs from the more common Duchenne MD. Collagen synthesis in FSH is normal whereas in Duchenne MD, collagen synthesis is elevated compared to controls. In early stages of FSH MD the activity of ribosomes for non-collagen synthesis is about three-fold higher than controls.

Protein synthesis in Becker MD also differs from the Duchenne-type even though both are X-linked. The concentration of ribosomes in the Becker form is lower than normal and lower than in Duchenne. Non-collagen protein synthesis is normal unlike Duchenne but collagen synthesis is increased as it is in Duchenne. Taken as a whole these experiments support the fact that these muscular dystrophies are inherited as separate diseases and thus are likely caused by defects in different gene products.

Protein synthesis determinations in 63 suspected carriers have been completed. Twenty of 28 carriers below 30 years of age showed an increase in collagen synthesis, though lower generally than that observed in patients with the disease. In carriers over 30 only 5 of 14 showed high collagen synthesis. Of 63 carriers, 42 were scored as carriers using the criteria of serum CPK, muscle histology, and protein synthesis. The reliability of carrier detection may be estimated at better than 90% when serum CPK, muscle histology, and protein synthesis determinations are all considered. The most immediate practical use of this work is in the detection of carriers of Duchenne MD. Pre-natal detection of the disease in the fetus may be possible.

Other investigators are seeking an explanation of pathology of genetic muscle diseases in terms of systemic membrane dysfunction. Dystrophies may reflect a genetic alteration of membrane composition which produces, in turn, altered ion conductance and consequent defective excitation coupling. As mentioned above, altered erythrocytes appear to offer some attractive possibilities with regard to diagnosis and carrier detection. Washed red cells from dystrophic mice and humans, when examined with the scanning electron microscope, exhibit severe deformation presumably owing to alterations in the membrane.

There is other evidence for genetically-produced changes in dystrophic red cell membranes. Electrophoretic examination of proteins from red cell ghosts indicates that the extrinsic protein, spectrin, is more easily lost on preparation of ghosts from dystrophic blood samples than in the normal case. This increased loss of a loosely bound protein may be related to increased membrane fluidity.

Ca^{2+} -stimulated ATPase of the red cell membrane, a relatively loosely bound protein, exhibits diminished activity in ghosts from dystrophic cells. The transport Na^{+} - K^{+} ATPase of ghosts is also significantly diminished in activity in ghosts from red cells from Duchenne dystrophic individuals and the kinetic properties of this protein are changed radically.

Another investigator is doing an analysis of the role of the lysosome and its complement of acid hydrolases in physiological and pathological muscle metabolism.

The sarcoplasmic reticulum is one of the most interesting specializations of the vacuolar apparatus. It was brought to prominence with its demonstrated role in excitation-contraction coupling. A few years ago another possible function was suggested. Electron dense materials in the lateral sacs of the S-R were reported and after staining for acid phosphatase the reaction product was particularly localized in the region of the triad, and a smaller amount

of the enzyme related to the longitudinal tubules. The investigator hypothesizes that the lysosomes (if they exist) are part of the sarcotubular system -- i.e. "sarcotubulolysosomal system." He believes that some of the muscle lysosomes can arise from the S-R when needed by an animal for degradation of muscle tissue.

The mechanism of muscle degeneration in disease states, tenotomy or disuse atrophy, denervation, vitamin E-deficiency in certain species, hyper-corticism, or breakdown during metabolic demand during protein-calorie restriction or starvation, remains to be elucidated. A common observation in muscle disease is the complete degeneration of a single muscle fiber, or segment of the fiber, lying alongside apparently normal muscle fibers. The first morphological change at the ultrastructural level in progressive muscle diseases, is dilation of the sarcoplasmic reticulum. There is the possibility of this tubular sleeve around the muscle fibril coalescing to segregate the fibril from the rest of the muscle, and turning into a large autophagic vacuole which may attain acid hydrolases by fusing with a lysosome. This is being studied.

Another project concerns an investigation of the metabolic interrelationships of selenium and vitamin E in the prevention of myopathies. Selenium and vitamin E deficiencies result in accumulation of calcium in lambs, and the understanding of how these two nutrients are involved in the proper metabolism of calcium could yield information regarding soft tissue calcification in humans. White muscle disease (WMD) in lambs resembles in many respects muscular dystrophy in humans and a better understanding of selenium in metabolism could yield information which could be used as a model system for studying other myopathies.

In another laboratory investigators are attempting to find mechanisms that control the activity and the localization of acetylcholinesterase (AChE) during muscle development and maturation, and, secondarily, to explore the nature of the defect in AChE regulation in inherited muscular dystrophy of the chicken. They are examining the ultrastructural localization of AChE in embryo muscle cultures and in muscles of normal and dystrophic chickens; of denervated and cultured muscle; comparing regulation of AChE in cultured muscle and nerve and studying the site and nature of the defect in AChE regulation in the dystrophic chicken.

One striking finding was quantifiable differences in AChE localization between single fibers of normal, denervated and dystrophic muscle. These workers have devised a microphotometric method to quantify AChE in single fibers. AChE of normal muscle fibers was arranged in a Poisson distribution within 50 microns to either side of the motor endplate. Dystrophic fibers had a similar distribution but activity was higher and extended to more than 300 microns to either side of the junctions. In contrast, denervated normal muscle fibers had a constant high level of AChE along the fibers except at the motor endplate where it was higher. It was as though AChE regulation was interrupted throughout denervated fibers and defective only around motor endplates in dystrophic fibers. Electrical stimulation reversibly reduced approximately 50 percent of muscle AChE activity.

Denervated twitch muscle AChE increased more than AChE from denervated multiple innervated muscle. AChE appeared in the plasma of birds with extensive denervation. Electrical stimulation studies in vivo and in vitro establish the importance of muscle activity in the control of AChE levels. The similarity of the regulation of AChE in muscle and nerve and the effect of curare on "induction" of AChE by Acetyl- β -methylcholine suggest that ion shifts and membrane excitation and not muscle contraction may be the important factor in the mechanism regulating AChE. The finding of release of AChE into plasma after denervation completes a series of experiments demonstrating AChE in plasma whenever AChE is high in muscle.

The finding that AChE is released from cultured nerves reopens the question of whether nerve, muscle or both put AChE at motor endplates. The localization of AChE around the motor endplate supports the idea of a defect in motor endplate region in dystrophy. The difference in localization of AChE between a myogenic defect (dystrophy) and a neural lesion (denervation) together with findings of AChE in human muscles raises the possibility of a future clinical test to distinguish some kinds of neuromuscular abnormalities.

MYASTHENIA GRAVIS

Myasthenia gravis is a neuromuscular defect affecting approximately 30,000 people in the United States alone. It is generally attributable to a failure of neurochemical transmission at the myoneural junction. It is probably due to either the inadequate release of acetylcholine from the nerve terminals or to a decreased sensitivity of the motor endplate to the transmitter. There is also evidence that MG may be an autoimmune disease resulting in antibody interference with neuromuscular transmission. Drug therapy in MG is based either upon increasing the available acetylcholine at the junction or upon the suppression of the autoimmune mechanism.

Modifications of this disease may occur with changes in the activity of the thyroid and tumors of the thymus are not infrequently a concomitant finding. The disease is of uncertain cause in which there is weakness without signs of neurodisorder, it has a special predilection for cranial muscles, it tends to vary in severity, and is partially relieved by cholinergic drugs.

Above the age of 40, men and women are affected equally often. Children born of myasthenic mothers are often affected by transient weakness. However, there is no clear evidence of a genetic pattern to the disease.

The onset is usually insidious without obvious cause. There is a typical expressionless face with drooping of the eyelids. There may be in addition a weakness of the ocular muscles. There may also be excessive fatigability of the muscles of the hand which can be demonstrated by the patient when he squeezes a dynamometer. A certain diagnosis may be obtained when injection of neostigmine or edrophonium results in a dramatic improvement in muscle strength for a short period of time.

The therapy of choice is neostigmine. Sometimes adrenal steroids are useful as a supplement in patients with severe myasthenia. Thymectomy has been used routinely in many clinics dealing with large numbers of patients with myasthenia. This is so despite the fact that there has never been a controlled matched prospective study of the value of the operation. However, there is some evidence of improvement after thymectomy, particularly in those cases where the patients have thymoma.

Involvement of the immune system in MG had previously been suggested by the occurrence of thymic hyperplasia, the presence of antibodies directed against muscle structural proteins, the presence of lymphocytes toxic to muscle tissue, and the beneficial effects of repeated lymphocyte drainage. In the past year, a number of different laboratories have produced paralysis in laboratory animals by the injection of purified muscle receptor protein. The animal model, similar to MG in pharmacological response to neostigmine and electromyographically, has been reproduced in rabbits, rats, goats, and monkeys.

The neuromuscular clinical research center at Duke University Medical Center, Durham, North Carolina has as one objective of its program to apply highly advanced electrophysiological techniques to the altered neuromuscular transmission of myasthenia gravis. Many studies have documented the involvement

of the neuromuscular junction in this disease but it is still not clearly resolved whether the primary site of pathology is the nerve terminal or the muscle endplate. Electrophysiological studies of the effects of myasthenic acetylcholine receptor antibodies on neuromuscular transmission in vitro together with analyses of the electrophysiological properties of the myasthenic neuromuscular junction should help define the site and mechanisms of impaired function. These researchers hypothesize that failure of neuromuscular transmission in myasthenia gravis is due to a change in the structure of the acetylcholine receptor which reduces its affinity for acetylcholine. The change in structure of the acetylcholine receptor is thought to result from action of an anti-acetylcholine receptor antibody which they believe they have identified and isolated from the serum of patients with myasthenia gravis. They are isolating the acetylcholine receptor using bungarotoxin which is known to react with the receptor selectively and irreversibly. Inhibition of the interaction of bungarotoxin with the isolated acetylcholine receptor will be used to demonstrate the presence of myasthenic immunoglobulin in serum. Several myasthenic patients are being followed to determine whether circulating antibody correlates with clinical state. This study of the myasthenic globulin reactions with receptor fraction is of great interest and has potential significance towards the understanding of this disease. Electrophysiologic studies are being conducted to determine the effects of serum and gammaglobulin preparations on neuromuscular transmission in myasthenic patients. In addition detailed analyses of neuromuscular transmission in myasthenia are being conducted in order to determine the sites of defects. Investigators in these laboratories as well as others have produced paralysis in laboratory animals by injecting purified muscle receptor protein. The animal model resulting is similar to myasthenia gravis in the pharmacological response to neostigmine as well as in their electromyographic reactions.

Work related to that being done in Durham is ongoing in one laboratory of the clinical research center for neuromuscular disease at Columbia University, New York City. Investigators are preparing acetylcholine receptor from mammalian muscle membrane in a search for anti-receptor antibodies in serum from myasthenic patients. Here too, alpha-bungarotoxin which binds specifically to the receptor is being used to identify these receptors. This work attempts to deal with two issues: Evidence one way or the other that the post-synaptic portion of the junction is affected in myasthenia and the other is that the defect is an immunological one. These techniques could apply to other immunologic studies such as the determination of whether receptor has antigenic properties in common with myofibril proteins and thymic elements. The work is important because it may provide evidence of an immunologic effect at the endplate in myasthenia.

In related work at the Salk Institute for Biological Studies in La Jolla, California, an investigator has been studying the acetylcholine receptor (AChR) protein at a molecular level to determine whether muscular weakness induced in animals by immunization with AChR is a valid model of myasthenia gravis (MG). Last year he worked to establish the validity of "experimental autoimmune myasthenia gravis (EAMG)," the name given to the disease induced in animals by immunization with AChR. He is also attempting to develop a sensitive assay for antibodies to AChR in the sera from MG patients, which will be further evaluated as a diagnostic test for this disease. Establishment

of EAMG as a valid model for human MG was achieved through fruitful collaborations with several workers at the Salk Institute and other institutions. In rats, EAMG was induced by a single injection of eel AChR. Dose response curves have been obtained for disease induction and antibody production.

An important discovery during the last year was development of a sensitive assay for antibodies to AChR from human skeletal muscle in the sera from MG patients. This method shows potential as an improved diagnostic test for MG. Antibodies to AChR from human muscle were found in 92 of 94 sera from MG patients tested, and not detected in any of 90 sera samples tested from normals and patients with other diseases. Although antibody titer was not closely related to disease intensity it was higher in MG patients with thymoma. Sera from twins with neonatal myasthenia contained antibodies to AChR at about 20% of the level found in their mother, suggesting that their transient disease was caused by antibodies received from the mother. Antibody titer decreased after treatment with steroids.

These immunological studies of AChR and the induction of an autoimmune disease by immunization with AChR first reported by Patrick and Lindstrom (Science, 180, 871, 1973) are now being conducted in many laboratories across the world. Validation of EAMG as a model for MG should allow development of improved therapy for this disease to be developed through studies of EAMG.

Another research group is studying acetylcholine metabolism at the synapse and neuromuscular junction and its response to disease. They use the neurotoxin of Naja naja siamensis as a marker for the acetylcholine receptor in crude and purified membrane fractions from mammalian skeletal muscle and from the electric organ of Torpedo Californica. They have prepared antibody to purified receptor in rabbits and in goats, and have reported a myasthenia gravis-like illness in these immunized animals. This line of work is to be extended to other proteins at the cholinergic synapse.

In another laboratory, workers are investigating mechanisms governing the desensitization of cholinergic receptors by quaternary ammonium compounds. More specifically, they are concerned with the role of Ca in receptor desensitization. Further, they are studying the action of a number of pharmacological and chemical agents which modify neuromuscular transmission with particular emphasis on morphological changes, receptor desensitization, and modification of prejunctional release mechanism. Results obtained over the last year indicate that postjunctional receptor desensitization can be produced by repeated application of carbamylcholine. The susceptibility of muscle fibers to production of postjunctional desensitization by the above means varies in a given muscle preparation.

During the past year another investigator has examined the presynaptic effects of three drugs used in the treatment of myasthenia gravis and myasthenic syndrome (germine-monoacetate, guanidine and neostigmine). The presynaptic effects of prolonged use of neostigmine were examined in the rat. Significant reductions in quantal release, mobilization activity and releasable store were found. These effects tend to counteract the known beneficial effects of the drug. Gernine-monoacetate had no effect on the presynaptic parameters but did result in a high frequency burst of endplate potentials following a

single nerve stimulus. Guanidine significantly increased quantal release, the mobilization rate and the probability of release. These effects may be due to a calcium site of action.

The studies further elucidate some of the properties associated with neuro-muscular transmission. They suggest that the mobilization process (replenishment of transmitter in the nerve ending) involves activity of microtubules. They also suggest that the amount of transmitter released per impulse is dependent on the rate of transmitter synthesis activity in the nerve ending. In the case of myasthenia gravis an impairment in mobilization and transmitter synthesis activity may be occurring. Accordingly the present results may help to reveal the major defects associated with this disease.

Other workers have shown that the spontaneous miniature endplate potentials (s-MEPP) of myasthenic muscle are about 1/5 normal although the ultrastructure of vesicles and terminals in general appears normal. In addition, it has been shown that the postsynaptic membrane of myasthenic muscle responds to acetylcholine with the same sensitivity as normal muscle. These studies suggest that the lesion may be presynaptic. Since the myasthenic miniature endplate potentials are of the same size as s-MEPPs, the investigators propose that the mechanism which normally synchronizes the release of several s-MEPPs to produce a classical MEPP is compromised in the myasthenic. Effects of botulinum toxin and tetanus toxins on MEPPs of myasthenic muscle (transmitter release) is of great interest since it has been proposed that myasthenia is an autoimmune problem. The possibility that thymus may release a toxin which blocks an excitation-secretion mechanism in the release of transmitter in a similar fashion to botulinum toxin is being explored.

Work on facilitatory effects of glucocorticoids on mammalian motor nerve function has been reported. Short-term, high-dose treatment with a glucocorticoid strongly enhances the neurally evoked post-tetanic potentiation (PTP). Since this PTP is generated by the terminals of the tonic motor nerves, this method provided an excellent means of locating and measuring the extent of the steroid action. Thus, various corticosteroids and a number of different dosing regimens were examined in order to define the most effective drug and an optimal schedule of administration. The glucocorticoids, triamcinolone and fluorocortisone were most potent in augmenting soleus PTP.

The facilitatory drug potentiation of twitch, as exemplified by edrophonium, is equally enhanced by glucocorticoids. This action very likely underlies the anti-myasthenic effect of comparable clinical regimens employing glucocorticoids or ACTH. There is presumptive evidence to indicate that the glucocorticoid regimen increases transmitter availability and release in motor nerve terminals. Thus, the anti-myasthenic effect could be explained by this steroid action on motor nerve.

Glucocorticoids are extensively used, and are especially effective in myasthenia gravis, and since little is known of their effects on synaptic transmission the present work may furnish important insights into corticosteroid actions on synaptic functions.

ANNUAL REPORT
For Period July 1, 1975 through June 30, 1976
Developmental Neurology Branch, Neurological Diseases Program
National Institute of Neurological and Communicative
Disorders and Stroke
National Institutes of Health

GENERAL SUMMARY

I. INTRODUCTION

The Developmental Neurology Branch (DNB) was created on August 26, 1975, within the Neurological Disorders Program of NINCDS. The Perinatal Research Branch became the Perinatal Research Section (PRS) within the Developmental Neurology Branch. The completion of the Comprehensive Plan for Analysis and Interpretation of Collaborative Perinatal Project Data continues to be a major objective of the DNB. As this objective is fulfilled, DNB will focus increasing effort on developing and implementing a program of research on the neurological basis of the developmental disorders of children including cerebral palsy, autism, mental retardation, learning and behavioral disorders, central nervous system birth defects, heritable muscle and neurological diseases, and minimal brain dysfunction. The PRS will provide for the archival and retrieval system of Collaborative Perinatal Project data and promote the appropriate use of this national data resource commensurate with the goals and mission of the NINCDS.

The Collaborative Perinatal Project (CPP) is a longitudinal multidisciplinary research effort which seeks leads to the etiologies of cerebral palsy, mental retardation, learning disorders, congenital malformations, minimal brain dysfunction, convulsive disorders and communicative disorders through studies which relate the events, conditions, and abnormalities of pregnancy, labor and delivery to the neurological and mental status of the children of these pregnancies as the child grows and develops. Data collection has been completed, and the major emphasis of the project has shifted to data analysis and interpretation for publication of reports on the CPP research findings. In order to accomplish this, a coordinated effort has been implemented to meet the basic data analysis objectives of the CPP. To provide a framework for this effort, a Comprehensive Plan for Analysis and Interpretation of Collaborative Perinatal Project Data has been approved and implemented.

II. DATA COLLECTION:

The Collaborative Perinatal Project was initiated in 1959 when the women began registering at each of the collaborating institutions during their pregnancies. Women continued to enroll in the study through December of 1965. The babies were delivered between 1959 and 1966 and received several examinations during their first year of life, at 3 years of age, and at 4

years of age. The 7-year extensive battery of examinations included a pediatric-neurological examination; a battery of psychological tests, including an IQ determination; and a visual screening test. By June of 1974, an assessment of speech, language and hearing development was completed on children eight years of age at five of the collaborating institutions. Completion of the eight-year examination terminated follow-up of CPP children. The last of these examination protocols has been edited, coded and the data punched and transferred to computer tape thus completing the basic data file of the CPP.

III. A COMPREHENSIVE PLAN FOR ANALYSIS AND INTERPRETATION OF COLLABORATIVE PERINATAL PROJECT DATA:

Broadly stated, the Collaborative Perinatal Project is concerned with the identification of prenatal factors that have sufficiently high association with adverse pregnancy outcome and subsequent neurological and mental development of the child to provide leads to the etiologies of the abnormalities and thus to the development of strategies for prevention and intervention. After a careful review of the objectives of the Collaborative Perinatal Project, the data available for analysis and the work in progress, it was recommended that major efforts in analysis and interpretation were needed in ten primary areas in order to meet the basic objectives of the project. Monograph reports in book form are planned in each of the following primary areas:

Cerebral Palsy (See Individual Project Reports -
Project Nos. Z01 NS 02059-04 DNB,
Z01 NS 02172-02 DNB)

Mental Retardation (See Individual Project Reports -
Project Nos. Z01 NS 02106-03 DNB,
Z01 NS 02172-02 DNB)

Communicative Disorders (See Contract Narrative
N01 NS-4-2326)

Visual Abnormality (See Individual Project Report -
Project No. Z01 NS 02107-03 DNB)

Convulsive Disorders (See Individual Project Report -
Project No. Z01 NS 02058-03 DNB)

Learning Disorders (See Individual Project Report -
Project No. Z01 NS 02108-03 DNB)

Minimal Brain Dysfunction (See Individual Project Report -
Project No. Z01 NS 02062-04 DNB)

Congenital Malformations (See Individual Project Report -
Project No. Z01 NS 02109-03 DNB)

Birthweight-Gestational Age Relationships (Prematurity) (See
Individual Project No. Z01 NS 02060-04 DNB)

Neuropathology, General Pathology and Placentology (See Contract
Narratives, Contracts N01-NS-3-2311 & N01-NS-3-2312)

IV. IMPLEMENTATION OF THE COMPREHENSIVE PLAN

Implementation of the Comprehensive Plan for Analysis and Interpretation of Collaborative Perinatal Project Data is carried out through teams of researchers, headed in each of the ten primary areas by a member of the Professional Staff Consultants of the Developmental Neurology Branch. On each team, there is a member of the Office of Biometry and Epidemiology staff, who participates fully in the development of the analysis. In addition, two members of the Production of Data Analysis unit, Perinatal Research Section, are assigned to each primary area to facilitate data processing. The assignments are as follows:

| <u>PRIMARY DATA ANALYSIS AREAS</u> | <u>BRANCH</u> | <u>OFFICE OF BIOMETRY</u> |
|---|--------------------------|---------------------------|
| Cerebral Palsy | Dr. K. B. Nelson | Dr. J. H. Ellenberg |
| Mental Retardation | Dr. S. H. Broman | Dr. P. W. Shaughnessy* |
| Communicative Disorders | Dr. P. J. LaBenz | Mr. D. Rubinstein** |
| Visual Abnormality | Dr. R. Feinberg | Miss E. C. Jackson |
| Convulsive Disorders | Dr. K. B. Nelson | Dr. J. H. Ellenberg |
| Learning Disorders | Dr. S. H. Broman | Dr. P. W. Shaughnessy* |
| Minimal Brain Dysfunction | Dr. P. L. Nichols | Dr. T. C. Chen |
| Congenital Malformations | Dr. N.C. Myrianthopoulos | Mr. D. Rubinstein** |
| Birthweight-Gestational Age Relationships (Prematurity) | Dr. Bill H. Williams | Miss E. C. Jackson |
| Neuropathology, General Pathology and Placentology | Dr. J. S. Drage | Dr. T. C. Chen |

In each of the ten primary areas, a program plan has been developed and approved which expands in detail on the summary statements in the Comprehensive Plan and gives a detailed approach to the analysis and identifies

*No longer with the Office of Biometry, but continues to serve as a Consultant in this area.

**No longer with the Office of Biometry after October 10, 1975.

major components. Written monthly reports are prepared and meetings held to record progress and identify problem areas. In order to facilitate the primary data analyses and/or complete major efforts well underway, additional analyses are to be completed in the following secondary areas:

Toxemia (See Contract Narrative, Contract N01-NS-3-2320)

Maternal Infection during pregnancy (See report by Infectious Diseases Branch, NINCDS)

Neonatal Hyperbilirubinemia (See Individual Project Report - Project No. Z01-NS-02112-03 DNB)

Maternal anesthesia-analgesia during labor and delivery
(See Individual Project Report - Project No. Z01-NS-02169-02 DNB)

Four-Year IQ (completed in FY 75)

Physical Growth and Development (Birth to Seven Years) (See Contract Narrative, Contract N01-NS-5-2308)

Twins (See Individual Project Report - Project No. Z01-NS-02109-03 DNB)

Genetic and Socio-economic Factors (See Individual Project Reports - Project Nos. Z01 NS 01514-10 DNB, Z01 NS 01857-07 DNB
Z01 NS 01754-08 DNB, Z01 NS 01274-12 DNB)

Drugs taken during pregnancy (See Contract Narrative, Contract N01-NS-2-2322)

Labor and Delivery (To be developed pending completion of Toxemia investigation)

V. SUMMARY OF WORK IN PROGRESS

In the cerebral palsy area, the preliminary screening of antecedent obstetric variables and early pediatric clinical manifestations with regard to their association with cerebral palsy has been completed. A listing of the conditions most strongly associated has been made and will form the basis for multivariate analysis. The analysis of demographic factors is now available and is in the process of assessment. Cerebral palsy at seven years is somewhat more frequent in boys than girls, and among whites than blacks. Ten percent of cerebral palsy is apparently caused by events occurring after the first month of life. Clearly handicapping cerebral palsy was present at age seven in 22-32/10,000 children.

In the mental retardation area, selected findings indicate that the incidence of severe mental retardation (IQ <50) was approximately one-half

of one percent, regardless of ethnic groups. Approximately 70 per cent of these severely retarded children had major neurological problems. Mild retardation (IQ 50-69) was more common among Black and Puerto Rican children than among White. Between 15 and 24 percent of the mildly retarded had major neurological problems. Signs of perinatal anoxia were associated with mental retardation. Infant psychomotor test scores at 8 months were good predictors of mental retardation, especially the severe category. I.Q. scores at age four were also good predictors of mental retardation as well as normal and superior intellectual performance at age seven. Mild retardation, but not severe, was associated with low socioeconomic status.

A group of children with both cerebral palsy and mental retardation were examined for perinatal risk factors. This double disability was present in 1/590 children in the CPP; of these 90 children half were institutionalized by age seven years. Among the 50 children whose deficits were not accounted for by anatomic, metabolic, or other known factors, the most striking relationships were found for neonatal characteristics; including (1) small size at birth, (2) difficulty with independent respiration, (3) low hematocrit and hemoglobin and (4) neonatal seizures.

Studies in the area of communicative disorders have provided analyses which disclose associations among speech, language and hearing (SLH) outcomes, and the relationships of these outcomes to variables in other study areas. SLH data were examined intensively for quality and other CPP variables were scrutinized for validity, reliability, redundancy and missing data. A total of 680 CPP variables were finally selected. In order to facilitate study of the relationships with these CPP variables, key SLH variables were identified and a number of composite indexes were constructed to serve as summary descriptors. A total of 27 SLH indexes and key variables emerged which were used to construct a correlation screen against the 680 preselected CPP variables. Multiple regression analyses were performed, using variables surviving the correlation screen. Data collected at birth and mental and motor scores at 8 months account for less than 8 percent of the variance in the SLH indexes at three years of age. Indexes on outcomes at eight years of age were also subjected to multiple regression analyses, adding the predictor variables of 4 year I.Q. and SLH indexes at three years. The 4 year I.Q. demonstrated pronounced effects. The 8-month variables contributed very little to any of the predictions, and three year indexes alone were not successful predictors of 8 year indexes.

In the area of visual abnormality, analyses of selected predictor variables using a point biserial correlation and a chi-square analysis of dichotomized variables produced numerous positive associations with defined visual defects at seven years of age. These two outputs currently are serving in a screening process to select variables for multivariate analyses.

In the convulsive disorders area, the preliminary program to screen antecedent obstetric variables and early clinical manifestations with

regard to seizure disorder diagnoses has been completed. The analysis of demographic factors and their impact on the incidence and risk of seizure disorders is now in the process of assessment. Approximately one in twenty children followed to the age of seven years had at least one seizure. About one-tenth of these children who had at least one seizure had active epilepsy by the age of seven. The rate was slightly higher in girls, and approximately equal in Whites and Blacks. The most common disorder is febrile seizures found in almost one in twenty-five of the CPP population. The risk of epilepsy is increased among children who have had febrile seizures, being highest in children who were suspect or abnormal in neurological development before any seizure. Clinical features of the febrile seizure were also of predictive value. Seizures occurring in the first month of life were associated with a relatively high rate of death as well as subsequent disability, including cerebral palsy.

In the learning disorders area, findings show that low achievers at age 7 showed fairly wide-spread cognitive deficits and behavioral deviations at age 4; also present at age 7 were deficits in visual-motor performance, verbal ability and short-term memory, and certain neurological soft signs. Indices of socioeconomic status and family structure were more strongly related to low achievement than were indices of physical development and medical status.

In the area of minimal brain dysfunction, a factor analysis was used to define four components of a possible syndrome: learning disabilities, hyperkinetic impulse disorders, social immaturity, and minor neurological problems. Children have been scored on these four components and correlation coefficients calculated with over 600 antecedent and concomitant variables. Many socioeconomic, maternal, neonatal, and developmental variables were shown to have significant associations with the component scores. Association among components is present, although most children who have problems in one area do not have problems in other areas.

The first four segments of the 11 segment congenital malformations analysis plan have been published. A basic data file has been completed to be used in the genetic analysis. This file combines genetic information such as family linkage and twin zygosity with various outcome variables such as cerebral palsy, congenital malformations, mental retardation, etc. Several strong associations have been detected between ABO or Rh blood types and congenital malformations. Analysis of multiple malformations indicates that 14 per cent represent known syndromes, 54 per cent primary single malformations with accompanying secondary, and 32 per cent remain unclassified.

In the birthweight-gestational age area the relationships of birthweight to placental weight, maternal education, socioeconomic index, gestation at registration, year of birth, institution, body length at birth and head circumference at birth have been analyzed. The particular relationships

of birthweight to placental weight and to gestational age with respect to survivors and non survivors have also been analyzed. The results of these studies are currently being compiled and comprise Phase I of the Birthweight-Gestational Age Study.

Studies in the area of pathology continued under two separate contracts, one for neuropathology and one for general and placental pathology. In the neuropathology area review of case material continues. Approximately 1300 cases have been completely reviewed and the findings coded. An appraisal of myelination in serially sectioned cases is underway, the sequence being related to other aspects of brain maturation, and to antecedent events. Other studies include a description of brain growth, temporal development of principle gyri and sulci, incidence of heterotopias in the cerebellum, pigment deposition in the pineal gland, and correlation of placental and neuropathological findings. In the general pathology area, diagnoses on the placentas have been completed, and the primary review of 49,200 cases also completed. Statistical analyses of these data and post mortem diagnoses continue in terms of relationships with other variables in the CPP.

In the toxemia area the analysis objectives of Phase IV of the contract dealing with the dynamic time-trend analysis of Project data relating to the diagnosis of all gravidas according to toxemia classification and the delineation of the constellation of factors related to the subsequent development of significant disease have been achieved and the required monograph report is being prepared. A tape containing the diagnoses has been supplied to the Developmental Neurology Branch for use in other studies which are assessing the neurological and mental status of the children.

"The First Year of Life" is a volume reporting on the frequency distribution of findings reported on CPP children during the first year of their lives. It will include information on birthweight-gestation distribution, bilirubin levels, age at hospital discharge, and distributions of various pathological findings detected during the nursery stay and during the first year of life. Of particular interest will be information regarding brain abnormality as detected during the nursery period. This volume is intended to serve as a general description of the CPP children during their first year of life and as a reference document for further in-depth studies. The analyses for this work have been completed. The document will be ready for publication this year.

The neonatal hyperbilirubinemia study has shown that intermediate levels of serum bilirubin have an adverse effect on subsequent neurological and mental development. Neonates have been studied in birthweight-gestational age categories, and by socioeconomic class for a variety of outcome measures including mental and motor assessments at age 8 months and a spectrum of neurological findings at one year. The manuscript of Phase I of this study is complete and has been submitted for clearance.

A study of the relationship between intelligence at four years of age, as measured by the Stanford-Binet Intelligence Scale, and a variety of factors

related to pregnancy, delivery, postnatal status, and environment, was published during Fiscal Year 1975. Broman, S.H., Nichols, P.L., and Kennedy, W.A.: Preschool IQ: Prenatal and Early Developmental Correlates. Hillsdale N.J.: Lawrence Erlbaum Associates (distributor, Halsted Press, John Wiley & Sons, New York) 1975, 360 pp.

Work in the area of maternal anesthesia-analgesia began during the Fiscal Year. Reports in the literature suggest that both anesthetics and analgesics decrease the quality of infant test performance and that the drug produced dysfunction is not short-lived. Preliminary CPP results indicate many differences in infant status and performance among anesthetic-analgesic groups.

A contract with an expanded workscope was implemented during the current Fiscal Year to provide detailed analysis of physical growth data on CPP children from birth to seven years of age. Maternal socio-economic status was found to be related to birthweight and hemoglobin levels. Physical growth is channel-wise from birth to a much greater extent than previously realized. Length of gestation was found to be an important parameter which should be taken into account in predicting subsequent growth patterns.

The study of the effects of maternal drug ingestion on perinatal deaths and congenital malformations continued during the Fiscal Year. A book manuscript is currently under review.

VI. CONTRACT DEVELOPMENT:

During this Fiscal Year the contract for microfilming CPP records was allowed to expire. A total of 19,000 records have been microfilmed leaving a balance of 42,000. The contractor experienced significant difficulties producing an acceptable microfilm product. A new contract is being sought and microfilming will resume before the end of this Fiscal Year.

A new contract was developed in the Physical Growth area, one of the secondary areas within the Comprehensive Plan. This contract will provide a comprehensive approach to the Physical Growth data. The contract was initiated on May 1, 1975.

The study of minor chromosomal variants, one of the segments of the congenital malformations analysis, is now being carried out under contract. The collection of chromosomal material from CPP children was sponsored by the National Institute of Child Health and Human Development, and the information on the congenital malformations is from the CPP. This contract was initiated on June 30, 1975.

VII. SUPPORT FUNCTIONS:

The Unit for Data Collection is responsible for receiving, editing, coding, filing, microfilming and storing of all project forms in accordance with a system designed to facilitate data retrieval. All in-house generated

Interdisciplinary Diagnostic Code forms have been processed through key-punching and all write-ins have been abstracted. The backlog of unfilled forms has been depleted and all forms have been filed in the case records. During the Fiscal Year the major efforts were concentrated on preparation of records for microfilming, editing the microfilm and correcting the Master Data File.

The Unit for the Production of Data Analysis has as its basic mission the processing and storage by digital computer of the medical research data collected in the CPP. The Unit provides computer data processing support to researchers in their analysis of the data. The Master File has been updated and resorted. All duplicate and invalid records have been removed from the file. This has resulted in faster processing at lower cost. Various, smaller study files have been created containing data of interest to researchers in the various areas. These files have also been kept updated. During the past year sixty-two requests for data processing were completed. A number of statistical packages have been rewritten for more efficient processing, or modified for specific CPP analysis needs.

VIII. OTHER MAJOR ACTIVITIES:

As an initial step in the development of a program on autism within the DNB, a Workshop on the Neurobiological Basis of Autism was held by the DNB, NINCDS, on February 26-27, 1976, at the NIH campus, Bethesda, Maryland. The goals of the Workshop were: (1) to formulate a working definition of autism to serve as a common basis for neurobiological research; (2) to provide assessment of the current status of neurobiological research on autism; (3) to identify promising research areas and provide recommendations for research activities to advance scientific knowledge of the neurobiological basis of autism. The Workshop was attended by 55 scientists from academic institutions and government. They included neurologists, pediatricians, psychologists, neurophysiologists, biochemists, speech, language and hearing specialists, and psychiatrists. Government agencies represented were the Bureau of Education for the Handicapped, the National Institute of Child Health and Human Development, the National Institute of Mental Health, and the Division of Developmental Disabilities, Rehabilitation Services Administration. The presentations and discussions centered around the following topics: definition, neuropsychology, language, neurophysiology (vestibular and evoked potential research) and neurochemistry. Plans are to publish the proceedings.

The Office of the Chief, DNB has been involved on a continuing basis throughout this Fiscal Year with the implementation of the Privacy Act of 1974 which went into effect on September 27, 1975. The Chief, DNB, serves as Privacy Act Coordinator for NINCDS. Systems of records were identified within NINCDS and notifications of these systems prepared and published in the Federal Register. Other involvements included training activities, planning activities, monthly reporting of access and disclosure, consultation with NINCDS staff, the development of guidelines for implementation, and the preparation of Privacy Act statements

to inform non-DHEW personnel who use our records of their responsibilities under the Act. A Privacy Act Annual Report was submitted for the Institute on April 21, 1976, which assessed the impact of the Act on our research functions.

Another major activity undertaken by the Office of the Chief during the Fiscal Year was the conducting of Clinical Research Panel review of contracts for the protection of Human Subjects to conform with revised DHEW regulations and NIH guidelines. The Chief, DNB, serves as Co-Chairman, Clinical Research Panel, for contracts. A panel was formed, meetings held, and documented in order to review NINCDS contract proposals with regard to their impact on the rights of patients and human volunteers to protection from risk, to informed consent, and to confidentiality.

CONTRACT NARRATIVE
Developmental Neurology Branch, NDP, NINCDS
Office of the Chief
July 1, 1975 through June 30, 1976

UNIVERSITY OF WISCONSIN: (N01-NS-2-2303)

Title: Genetic-odontometric study of pre and neonatal growth

Contractor's Project Director: Richard Osborne, Ph.D.

Current Annual Level: No funding -- see Proposed Course of the Contract below

Objectives: To correlate the incidence and prevalence of dental and facial abnormalities with neurological defects, congenital abnormalities and other disorders of childhood.

Methodology: Dental casts and photographs of teeth will be obtained on about 2500 Collaborative Project children who are scheduled for routine seven and eight year examinations at selected participating institutions, and on children rescheduled because of known complications during their prenatal and neonatal periods of study.

Current Status: Collection of casts and film on 2217 children has been completed. All dental measurements have been taken and discrepancies have been resolved. All observations and measurements have been put on tape. It appears that over 20 percent of children in this sample have dental abnormalities.

Significance to the Program: The study of dental abnormalities is expected to yield an index of stress during prenatal development.

Proposed Course of the Contract: The contract expired August 31, 1975. No further extension of the contract is anticipated. A final report has not yet been received.

CONTRACT NARRATIVE
Developmental Neurology Branch, NDP, NINCDS
Office of the Chief
July 1, 1975 through June 30, 1976

JOHNS HOPKINS UNIVERSITY (N01-NS-2-2321)

Title: Ancillary Studies

Contractor's Project Director: Janet B. Hardy, M.D.

Current Annual Level: \$77,441.00

Objectives: Using NINCDS Collaborative Perinatal Project data and data unique to the Johns Hopkins Collaborative Perinatal Study, the contractor will conduct studies in the following areas:

- a. Perinatal infections.
- b. Problems in communication.
- c. Growth and development of the child in an inner-city population.
- d. The psychological, biological and environmental factors relating to I
- e. The etiology and characterization of seizures.
- f. The effect of maternal smoking on the fetus.
- g. The toxicity of low levels of bilirubin.

The contract has been amended to include the following:

- 1.) Full participation by the Project Director in the analysis and interpretation of the data output from the birthweight-gestational age study to result in a manuscript suitable for publication as a monograph.
- 2.) Full participation by the Project Director in the analysis and interpretation of the data output of Phase I from the hyperbilirubinemia study.

Major Findings: See the following individual project reports.

- 1.) Birthweight-gestational age. Project No. Z01 NS 02060-04 DNB
- 2.) Hyperbilirubinemia. Project No. Z01 NS 02112-03 DNB
- 3.) The First Year of Life. Project No. Z01 NS 02052-04 DNB

Course of Contract: June 28, 1972 through June 27, 1976

Publications:

Hardy, J. B.: Birth weight and subsequent physical and intellectual development. N. Eng. J. Med. 289:973-974, 1973.

Hardy, J. B. and Mellits, E. D.: Does maternal smoking during pregnancy have a long-term effect on the child? Lancet. 2:1332-1336, 1972.

Hardy, J. B.: Clinical and developmental aspects of congenital rubella. Arch. Otolaryngol. 98:230-236, 1973.

Wolff, S. M.: The ocular manifestations of congenital rubella. A prospective study of 328 cases of congenital rubella. J. Pediatr. Ophthalmol. 10:101-141, 1973

Welcher, D. W., Wessel, K. W., Mellits, E. D. and Hardy, J. B.: The Bender-Gestalt test as an indicator of neurological impairment in young "inner city" children. Percept. Mot. Skills. 38:899-910, 1974.

Hardy, J. B.: Congenital rubella. In Ryan, S. J., Jr. (Ed.): Eye and Systemic Disease. New York, Grune & Stratton, 195-204, 1974.

Hardy, J. B.: Rubella and congenital rubella. Current Therapy. W. B. Saunders Company, 57-60, 1974.

CONTRACT NARRATIVE
Developmental Neurology Branch, NDP, NINCDS
Office of the Chief
July 1, 1975 through June 30, 1976

BOSTON UNIVERSITY (N01-NS-2-2322)

Title: Maternal Drug Ingestion and Fetal Abnormalities

Contractor's Project Director: Dennis Slone, M.D.

Current Annual Level: \$143,550.00

Objectives: Using NINCDS Collaborative Project data, the objective of the contract is to conduct a research program on maternal drug ingestion and fetal abnormalities. The study will describe maternal drug utilization patterns, provide for a rapid evaluation of drug teratogenicity, report on the relationship between Dilantin and selected congenital malformations, investigate the possible teratogenic effects of virus vaccines, and investigate possible associations between drugs and congenital cardiac malformations.

Major Findings: A manuscript in book form has been submitted to the Developmental Neurology Branch, entitled "Birth Defects and Drugs in Pregnancy". This manuscript is currently under review. It represents the major product of this contract. Thirty-four chapters are included. Chapters dealing with specific drug categories are as follows:

Analgesics and Antipyretics; Antimicrobial and Antiparasitic Agents; Immunizing Agents; Antinauseants, Antihistamines, and Phenothiazines; Sedatives, Tranquilizers, and Antidepressant Drugs; Drugs Affecting the Autonomic Nervous System; Anesthetics, Anticonvulsants, Muscle Relaxants, and Stimulants; Caffeine and Other Xanthine Derivatives; Diuretics and Drugs Taken for Cardiovascular Disorders; Cough Medicines, Drugs Taken for Gastrointestinal Disorders; Hormones, Hormone Antagonists, and Contraceptives; Inorganic Compounds and Certain Vitamins; Diagnostic Aids, Technical Aids, and Rare Drugs.

Course of Contract: June 28, 1972 through June 27, 1976

Publications:

Shapiro, S., Slone, D., Hartz, S. C., Rosenberg, L., Siskind, V., Monson, R. R., Mitchell, A. A., Heinonen, O. P., Idanpaan-Heikkila, J., Haro, S. and Saxen, L.: Anticonvulsants and parental epilepsy in the development of birth defects. Lancet. 1:272-275, 1976.

CONTRACT NARRATIVE
Developmental Neurology Branch, NDP, NINCDS
Office of the Chief
July 1, 1975 through June 30, 1976

PENNSYLVANIA STATE UNIVERSITY, M.S. HERSHEY MEDICAL CENTER (NO1-NS-3-2311)

Title: Analysis of General Pathology and Placental Pathology of the Collaborative Perinatal Project Data.

Contractor's Project Director: Richard L. Naeye, M.D.

Current Annual Level: \$136,412.00 extended 6 months without additional funding.

Objectives: This contract is in two parts. One part concerns the general pathology and the other part the placental pathology.

The Objective of the general pathology analysis is to review and classify deaths as to the cause of death, factors contributing to death, and other associated factors. The pathologist is making an assessment of morphologic diagnoses using Collaborative Perinatal Project (CPP) protocols, magnetic data tapes and histologic tissues and slides. An analysis will be completed in the following areas: 1) demographic characteristics (age, race, marital status and others deemed pertinent to a pathology-oriented analysis) of women to develop possible associations with stillbirth, neonatal, and later death; and other classifications of specific pathological or anatomical findings deemed relevant to demographic characteristics. 2) maternal conditions and events of pregnancy, labor and delivery to test for associations with stillbirth, neonatal, and later deaths, and with specific pathological and anatomical findings or groups of findings thought by the pathologist to need exploration. For neonatal and later deaths, data obtained on the child prior to death will be examined. Such analysis will include specific conditions; such as, hyaline membrane disease, erythroblastosis, congenital malformations, and sudden unexplainable death. 3) organ growth rates, including the quantitative extent to which a variety of reported maternal conditions and events may modify or contribute to altered organ growth rates.

The Objective of the placental pathology analysis is to review and classify the various placental findings as reported on study protocols by using the protocols, magnetic data tapes, and placental microscopic slides and tissues. Through such a review, the pathologist will be expected to define and create additional classifications of placental pathology. Analyses will be completed on 1) demographic characteristics and maternal conditions and events of pregnancy, labor and delivery by the classical and innovative pathological and anatomic categories established by the pathologist; and 2) birthweight-gestation classifications by placental weight, placental pathology and pregnancy outcome.

Major findings:

Coding of diagnoses on the placentas has been completed. Efforts to find and apply appropriate statistical models to the data have revealed that

Contract No.N01-NS-3-2311

a log logistic model is satisfactory and six disease processes have been analyzed in terms of selected CPP variables. Substantial progress has also been made in analyses of postmortem diagnoses in terms of CPP variables.

Course of Contract: June 1, 1973 through December 31, 1976.

CONTRACT NARRATIVE
Developmental Neurology Branch, NDP, NINCDS
Office of the Chief
July 1, 1975 through June 30, 1976

CHILDREN'S HOSPITAL MEDICAL CENTER, BOSTON, MASSACHUSETTS (N01-NS-3-2312)

Title: Combined Neuropathologic and Epidemiologic Study

Contractor's Project Director: Floyd H. Gilles, M.D.

Current Annual Level: \$206,145.00

Objectives: The contract will analyze the neuropathology collection of the Collaborative Project (CPP). An estimate of the quality of the material and a catalogue of gross brain abnormalities will be prepared. Plots of fetal brain weight of grossly normal brains against estimated gestational age, utilizing a Gompertz function, will be made and an analysis will be made relating events of pregnancy, labor, and delivery. A comparison will be made of rate of brain weight acquisition in utero to rate of brain weight acquisition after birth as a function of total (gestational plus survival) age. A study will be made of intracranial hemorrhage including topography of hemorrhage. A study will be done on the risk factors associated with perinatal telencephalic leucoencephalopathy. A study of cerebral necrosis is to be completed which would include criteria of necrosis in the perinatal brain, and an evaluation of selected risk factors in relation to subclassification of neuronal and white matter necrosis.

Major Findings: Approximately 1300 cases have been completely reviewed. This includes a review of the gross features of the brains, from either kodachromes or black-white photos, and an analysis of several hundred histological features of those cases with adequate tissue preservation. The findings have been coded and transferred to IBM punch cards. An appraisal of myelination in about 360 serially sectioned cases available has been undertaken. The sequence of myelination is being related to other events of maturation determinable in the brain; e.g., sulcation and gyration. This information also is being synthesized with historical data; e.g., parity, gestational age, birth weight, brain weight. Cross-tabulations of the myelination systems have been made. The brain growth study is being completed. The description of brain growth by a Gompertz curve is being compared to that described by a polynomial evaluation. The neuropathological features of cases falling outside the confidence limits of these curves will be checked to determine if those features are restricted to the outlying cases. The incidence of heterotopias in the cerebellum is being studied. Approximately 500 cases have been found to have heterotopic rests in either the vermis or the hemispheres, or in both sites. The deposition of pigment in the pineal gland is being studied with reference to age. The pigment has been stained with several metallic stains and has been found to be melanin.

Course of Contract: June 1, 1973 to June 30, 1976

CONTRACT NARRATIVE
Developmental Neurology Branch, NDP, NINCDS
Office of the Chief
July 1, 1975 through June 30, 1976

BETH ISRAEL HOSPITAL, BOSTON, MASSACHUSETTS (N01-NS-3-2320)

Title: A Dynamic Time Trend Analysis of Collaborative Perinatal Project Data on Patterns of Blood Pressure, Edema and Proteinuria (Toxemia) as Related to Pregnancy Outcomes.

Contractor's Project Director: Emanuel A. Friedman, M.D.

Current Annual Level: \$88,340.00 was extended 6 months without additional funding.

Objectives:

The objective of the "Toxemia of Pregnancy" contract is to establish a precisely-defined toxemia classification by an analysis and interpretation of Collaborative Perinatal Project (CPP) data pertinent to the clinical diagnosis of toxemia: blood pressure, proteinuria and edema. The objective of the CPP is to provide leads to the etiologies of neurological and mental abnormalities of children through a study of the conditions and events of pregnancy, labor and delivery. Toxemia is one major abnormality of pregnancy which is known to have an increased risk to the fetus of death or premature birth with attendant risk to the survivors. However, toxemia has been vaguely and inconsistently defined and this research effort is intended to classify the varying degrees of toxemia so that each precisely-defined degree of toxemia can be assessed for a possible etiological relationship with neurological and mental abnormality of the child.

Major Findings:

The following is excerpted from the overall summary of Progress Report No. 8 of the contract, dated March 1, 1976: With the exception of the required monographic report, all the objectives of Phase IV of the contract dealing with the dynamic time-trend analysis of Project data relating to the diagnosis of all Project gravidas according to toxemia classification and the delineation of the constellation of factors related to the subsequent development of significant disease have been achieved. The diagnostic classification derived in Phase III from the material evolved in Phases I-III has been applied to the formal retrospective diagnosis of all 58,806 Project gravidas for purposes of assigning critical and meaningful diagnoses. Additionally, we have been able to define a series of possibly relevant associated and contributory factors in personal and family history, actuarial information, findings on physical examination and laboratory study, submitting each variable from the total list of variables previously tabulated to statistical testing for significance thereby nominating those shown to be associated with the development of one or more of the newly-defined toxemia disorders for subsequent multivariate analysis. Discriminant analysis was applied to

those 53 variables determined to contribute significantly to the development of toxemia. By this method we succeeded in delineating those factors that are clearly related to the subsequent development of significant disease, thus identifying the gravida-at-risk for each disorder in the toxemia classification. Furthermore, we have examined the new classification system as it relates to toxemia diagnoses previously entered in the Project data files. Finally, we have built on the extensive knowledge and experience of the Boston Collaborative Drug Surveillance Program as related to drugs administered to Project gravidas within the new toxemia classification system, with special attention addressed to diuretics, antihypertensives, narcotic-analgesics, anticonvulsants and antidiabetic agents.

Course of Contract: June 25, 1973 through June 30, 1976.

CONTRACT NARRATIVE
Developmental Neurology Branch, NDP, NINCDS
Office of the Chief
July 1, 1975 through June 30, 1976

UNIVERSITY OF MINNESOTA (NO1-NS-4-2326)

Title: Analysis of Speech, Language and Hearing Deficits to Facilitate Prevention, Diagnosis and Treatment

Contractor's Project Directors: Frank M. Lassman, Ph.D., and
Robert O. Fisch, M.D.

Current Annual Level: \$243,547.00

Objectives: Data collected in the Collaborative Perinatal Project (CPP) have been analyzed to disclose associations among speech, language and hearing (SLH) findings, and the relationships of these findings to variables in other areas of study. These include variables relating to pregnancy, labor and delivery, family characteristics and the physical, mental and behavioral characteristics of the children. The associations among variables have been studied with an aim toward providing clues to the etiology of communicative disorders, and toward clinical application and prediction as well. Results of the analyses and the interpretation of their significance and applicability are being made available by publication as a monograph.

The SLH data were examined for quality in terms of availability, reasonableness of values and stability. Examiner variability was evaluated on the basis of test-retest results obtained from the CPP quality control program. Institutional variability was studied with particular emphasis on two data gathering institutions which used a non-standard sampling method at the 3-year level. In general, the quality of most SLH variables appears satisfactory with regard to the parameters used. The CPP variables were scrutinized repeatedly by consultants and CPP staff for validity, reliability, redundancy and missing data. A final list of 680 CPP variables was selected.

The cohort chosen for study included all White and Black core-study births (excluding walk-in registrants), examined within the age limits specified in the CPP protocol. Comparisons were made of the populations who attempted either, both or none of the SLH examinations in terms of the SLH variables and the CPP variables selected. Intercorrelations were calculated for the 3YR SLH variables and the 8YR SLH variables, using data on the population examined at both ages (3YR/8YR). These populations were described statistically by race, sex and socioeconomic index (SEI), by institution and in total. They appear similar in every respect, one to another and to the population not taking either SLH examination.

In order to facilitate study of the relationships with CPP variables, key SLH variables were identified and a number of composite indexes were constructed to serve as summary descriptors. SLH variables were evaluated by consultants for acceptability as potential components of indexes, and were

combined according to criteria specified by them. Frequency distributions and descriptive statistics were generated for each index, and inter-correlations were obtained among indexes and between indexes and SLH variables. Additionally, the method of Principal Components was used to obtain weightings for construction of another set of indexes. These were correlated with indexes constructed by the consultant panel. Three other more comprehensive indexes proposed by a previous task force also were subjected to the same evaluative process. A total of 27 SLH indexes and key variables emerged, and these were used to construct a correlation screen against the 680 CPP variables selected earlier. Multiple regression analyses using the earliest collected variables surviving the correlation screen yielded equations with varying predictive abilities. Correlations of residuals yielded no potential variables as additions to the multiple regression analyses.

Major Findings: Two multiple regression analyses were performed using the 3YR SLH indexes as outcomes. One used data at birth, and the other used 8-month mental and motor scores. In general, these predictors accounted for less than 8% of the variance. The better predictors were for 3YR Articulation, Intelligibility, Language Comprehension, and Sentence Complexity. Addition of the 8-month data did not materially improve prediction.

Five multiple regression analyses were done using the 8YR indexes as outcomes. In addition to the two sets of predictor variables used for 3YR outcome, the 4YR IQ and 3YR indexes were used in various combinations. Best predictions occurred for 8YR Word Identification (.75), Concept Development (.69), Written Communication (.61), Language Production (.54), Language Comprehension (.51), Auditory Memory (.49) and Articulation (.45).

The 4YR IQ demonstrated (by means of large Beta coefficients) pronounced effects in almost every multiple regression in which it was entered. The 8-month variables contributed very little to any of the predictions, and 3YR indexes alone were not successful predictors of 8YR indexes. A combination of variables at birth and 3YR indexes performed slightly better in predicting more than half the 8YR outcomes than did a combination of birth variables with 8-month scores and 4YR IQ.

Course of Contract: June 29, 1974 through June 28, 1976. Extension of time will be requested for completion, but with no additional funding. Considerable rerun of data was required to purge contaminants and caused delay in completion of analyses, particularly those related to some 18 ancillary studies.

CONTRACT NARRATIVE
Developmental Neurology Branch, NDP, NINCDS
Office of the Chief
July 1, 1975 through June 30, 1976

UNIVERSITY OF MICHIGAN (N01-NS-5-2308)

Previous Contract Number: N01-NS-2-2320

Title: Physical Growth Analysis

Contractor's Project Director: Stanley M. Garn, Ph.D.

Money Allocated: \$74,562.00

Objectives: To develop the physical growth measurement data on the 40,000 children examined within the framework of the Collaborative Perinatal Project (CPP). Specifically:

1. Develop for body weight, length and head circumference, a set of tabular, percentile, normative presentations of (a) size-for-age, (b) increments of size for age-interval and (c) size-for-size for age, for (1) whites, (2) blacks and (3) Puerto Ricans, and separately for (A) boys and (B) girls, comprising 3x3x2 types of tables, and approximately 200 in all, including those for (1) all live single births, (2) all term infants, (3) various socio-economic groupings and a constant-income grouping, (4) various birthweight categories including constant birthweight, and (5) simplified tables showing, the effects of socio-economic status, birthweight and other variables, including race corrected for socio-economic status. This set of tables is largely intended as a reference document for the Collaborative Perinatal Project.
2. Develop a set of summary tabulations and reports, directed to the major pediatric and growth-related users, complete with narrative and graphs, with the purpose of providing in the professional literature both an account of major substantive findings, and an in-the-literature account of the major data based along lines described in 1, but simplified as necessary.

Major findings: 1) Effect of maternal socio-economic status on birthweight and hemoglobin levels; 2) effect of birthweight on subsequent growth; i.e. physical growth is channelwise to a much greater extent than realized previously; and 3) gestational length should be taken into account in predicting subsequent growth patterns.

Significance to the Program: The above findings are new and important to the pediatric community as well as to physical anthropologists. Studies will be done delineating altered growth patterns in children with neurological problems.

Proposed Course: Prepare detailed analyses of the effects on physical growth data of socio-economic variables, birthweight variables, family size and parity, gestation variables, including various restrictions for gestation length, effects of race, family-line effects, channelwise progression (canalization), and the effects of exclusions for normality in final publication form.

and to be submitted for publication as results allow. In addition the following subgroups of medically abnormal children are to be examined for growth patterns which may be significantly different from those of the above "normals": (1) seizure disorders, (2) cerebral palsy, (3) congenital heart diseases, (4) microcephalics, (5) macrocephalics, (6) mildly mentally retarded, (7) severely mentally retarded, (8) high intelligence, (9) mongols, (10) sickle cell disease, (11) chronic respiratory disease (including respiratory distress syndrome and asthmatics), (12) congenital viral syndromes (including rubella), (13) selected metabolic and endocrine disease, (14) all medical exclusions as one group, and (15) a few additional selected disease groups which may be expected to have altered growth patterns.

Course of Contract: May 1, 1975 through April 30, 1977.

CONTRACT NARRATIVE
Developmental Neurology Branch, NDP, NINCDS
Office of the Chief
July 1, 1975 through June 30, 1976

UNIVERSITY OF COLORADO (N01-NS-5-2326)

Title: Congenital anomalies and chromosome variation

Contractor's Project Director: Herbert A. Lubs, M.D.

Current Annual Level: \$34,157.00

Objectives: To determine if there is a significant association between minor chromosomal variants and congenital malformations, and the possible mechanism for such association.

Methodology: The study will utilize chromosomal data on approximately 10,000 children from the Collaborative Perinatal Project, developed with conventional and banding techniques by a team of scientists at the University of Colorado Medical Center, and data on congenital malformations which occurred among these same children, developed at the Developmental Neurology Branch, NINCDS. See Project No. Z01 NS 02109-03 DNB.

Current status: The computer and programming work for a first round of analysis has been completed. Cross tabulations have been produced and tested by chi-square tests. A few significant associations have been noted and these are now being subjected to special analyses.

Significance to the Program: The study is expected to provide some insight into the etiology of congenital malformations.

Proposed course: The contract took effect on June 30, 1975 and expires on June 29, 1976. No extension of the contract is anticipated.

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| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER <div style="text-align: right;">Z01-NS-01163-14-DNB</div> |
| PERIOD COVERED <div style="text-align: center;">July 1, 1975 through June 30, 1976</div> | | |
| TITLE OF PROJECT (80 characters or less) <div style="text-align: center;">Selected Maternal Factors and Congenital Cardiovascular Anomalies</div> | | |
| NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT <div style="display: flex; justify-content: space-between; margin-top: 20px;"> <div>PI: L. Bajda</div> <div>Medical Consultant</div> <div>DNB, NINCDS</div> </div> | | |
| COOPERATING UNITS (if any) <div style="text-align: center;">None</div> | | |
| LAB/BRANCH <div style="text-align: center;">Developmental Neurology Branch</div> | | |
| SECTION | | |
| INSTITUTE AND LOCATION <div style="text-align: center;">NINCDS, NIH, Bethesda, Maryland 20014</div> | | |
| TOTAL MANYEARS: <div style="text-align: center;">0.4</div> | PROFESSIONAL: <div style="text-align: center;">0.4</div> | OTHER: <div style="text-align: center;">0.0</div> |
| SUMMARY OF WORK (200 words or less - underline keywords) | | |
| <p> This investigation into significant maternal factors related to pregnancy outcome at high "risk" for congenital heart disease uses data from records of the longitudinal epidemiologic <u>Collaborative Perinatal Project</u>. Observations on some 55,000 women provide "control" information for analysis of selected variables in the records of about 230 CPP women who delivered infants having a specific confirmed <u>congenital cardiovascular anomaly</u> diagnosed at any time thru seven years of age. Final correlations are under way. </p> | | |

Project Description

The Study has as its primary objective an epidemiologic investigation of relationships between maternal conditions and congenital cardiovascular anomalies. Identification of conditions putting the child "at risk" are sought.

Additional objectives include relating early signs of cardiac abnormality to cardiac diagnosis, growth, and mental status at 7-8 years of age as well as at intermediate levels. Emphasis on clinical attributes of the congenital heart case at various ages as well as the maternal history involved may provide a ready guide to the optimal care of the child.

Methodology

Study records from the Collaborative Perinatal Project (approximately 55,000 population) provide the data. Case number print-outs for children diagnosed as suspect or definite cardiacs on the one-year and seven-year summaries are used as indicators for the records searched for pre-selected maternal variables. After tabulation, analysis and comparison with computer provided control data, the use of statistical techniques should present a maternal "profile" for the infant "at risk" for congenital cardiovascular disease.

Major Findings

Presentations of preliminary findings on an initial sample of 82 and then 112 definite congenital cardiac cases emphasized the need for a larger study group in order that the maternal factors could relate to a specific diagnosis. General findings included a definite preponderance of mothers over 30 years age in both races after eliminating known chromosomal aberrations. There were also a greater than expected number of gravida with systemic disease complications and prior pregnancy loss. Underway is a larger analysis using 231 cases with specific confirmed congenital cardiac anomalies.

Significance to Biomedical Research

Use of epidemiological techniques to reveal source of disease is well proven. It is anticipated that the resultant guides to prevention of that once very crippling condition called "congenital heart" are at hand. To that extent to which the clinician and basic scientist can apply the findings in today's sociological setting will the significance of this study become apparent.

Proposed Course

Continuation of the above methodology and presentation of results to the primary care physicians wherever possible.

Publications: None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE
PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF
HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 01184-14 DNB

PERIOD COVERED

July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Population Dynamics of Tay-Sachs Disease and Other Sphingolipidoses

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER
PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: N.C. Myrianthopoulos

Research Geneticist

DNB NINCDS

COOPERATING UNITS (if any)

Dr. D. Gröschel, University of Texas

LAB/BRANCH

Developmental Neurology Branch

SECTION

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:

.05

PROFESSIONAL:

.05

OTHER:

.00

SUMMARY OF WORK (200 words or less - underline keywords)

The objective is to confirm experimentally the epidemiologic finding that the selective advantage of the TSD heterozygote is due to possible protection of the heterozygote from tuberculosis. Current experiments measure the phagocytic activity of mouse macrophages for mycobacteria in the presence and absence of GM₂ ganglioside.

Project Description:

Objectives: To confirm experimentally the epidemiologic finding that the selective advantage of the Jewish TSD heterozygote is due to possible protection of the heterozygote from tuberculosis.

Methodology: The experimental design is to measure the rate of growth of the mycobacterium tuberculosis in media with and without hexosaminidase A, and the rate of infection by the mycobacterium of tissues with and without lipid accumulation. Experiments are now in progress in which mouse macrophages are fed GM₂ ganglioside in vivo and in vitro, and the phagocytic activity for mycobacteria measured.

Major findings: None

Publications: Myrianthopoulos, N.C. and Melnick, M.: Tay-Sachs disease: a genetic-historical view of selective advantage. In: Tay-Sachs Disease: Screening and Prevention. New York, National Foundation, in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE
PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF
HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 01274-12 DNB

PERIOD COVERED

July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Genetic Bases of Neonatal Reflexes

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER
PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: A.F. Naylor
OTHER: N.C. Myrianthopoulos

Research Geneticist
Research Geneticist

DNB NINCDS
DNB NINCDS

COOPERATING UNITS (if any)

None

LAB/BRANCH

Developmental Neurology Branch

SECTION

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:

.02

PROFESSIONAL:

.02

OTHER:

.00

SUMMARY OF WORK (200 words or less - underline keywords)

Neonatal reflexes (suck, rooting, palmar grasp, plantar grasp, Moro, etc.) are usually tested as signs of neurological and general well-being. A study has long been planned as to whether genetic variables may be sometimes responsible for absence of specific reflexes. A data file under construction which contains genetic and background information will be enlarged to include data on neonatal reflexes so that the investigation can finally be carried out.

Project Description:

Objectives: To investigate the validity of regarding the suck, rooting and other neonatal reflexes as genetic entities.

Major findings: An initial set of cases retrieved for absence of one or more of these reflexes was reviewed and seemed to have high frequencies of various kinds of trauma whose base line frequencies were unknown.

Proposed course: To place limits on the frequencies of losses of suck, rooting, palmar grasp, plantar grasp and Moro reflexes because of mutation or segregation at gene loci specifically affecting manifestation of these reflexes.

Completion of the Variable and Family Linkage Files makes practical the reactivation of this project along proper lines. Base populations can be selected for general health, especially neurological, and frequencies of isolated absence or weakness of single neurological signs can be tested. Active work on this project will be undertaken when most current tasks have been carried out.

Publications: None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE
PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF
HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 01276-12 DNB

PERIOD COVERED

July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Sequential Aspects of Occurrence of Spontaneous Abortion in Family Histories

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER
PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: A.F. Naylor

Research Geneticist

DNB NINCDS

COOPERATING UNITS (if any)

D. Warburton, College of Physicians and Surgeons at Columbia University

LAB/BRANCH

Developmental Neurology Branch

SECTION

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:

.02

PROFESSIONAL:

.02

OTHER:

.00

SUMMARY OF WORK (200 words or less - underline keywords)

The objective of this study is to relate the risk of spontaneous abortion to maternal age and prior reproductive experience. A special point under investigation is whether apparent age effects are explicable by a tendency for intrinsic habitual aborters to remain in the reproductive population longer in attempts to compensate for unsuccessful pregnancies. Also conditional risks have been estimated.

Project Description:

Objectives: To relate the risk of spontaneous abortion to maternal age and prior reproductive experience. A special point under investigation is whether apparent age effects are explicable by a tendency for intrinsic habitual aborters to remain in the reproductive population longer in attempts to compensate for unsuccessful pregnancies. Also conditional risks have been estimated.

Proposed course: A manuscript arguing that age effects are real but laying emphasis on the decided importance of parity effects has been published. Naylor, A.F.: Sequential aspects of spontaneous abortion: Maternal age, parity and pregnancy compensation artifact. Soc. Biol. 21:195-204, 1974. Dr. Warburton is to begin a draft of the second manuscript which will deal with conditional risks of spontaneous abortion on the basis of tables and analyses which have been in existence for some time.

Publications: None

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| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER <div style="text-align: right; font-weight: bold;">Z01 NS 01514-10 DNB</div> |
| PERIOD COVERED <div style="text-align: center; font-weight: bold;">July 1, 1975 to June 30, 1976</div> | | |
| TITLE OF PROJECT (80 characters or less) <div style="text-align: center; font-weight: bold;">Record Linkage of Relatives Registered in the Collaborative Study</div> | | |
| NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT <div style="display: flex; justify-content: space-between; margin-top: 20px;"> <div style="width: 30%;"> PI: A.F. Naylor OTHER: N.C. Myrianthopoulos </div> <div style="width: 35%;"> Research Geneticist Research Geneticist </div> <div style="width: 30%; text-align: right;"> DNB NINCDS DNB NINCDS </div> </div> | | |
| COOPERATING UNITS (if any) <div style="text-align: center; font-weight: bold;">None</div> | | |
| LAB/BRANCH <div style="text-align: center; font-weight: bold;">Developmental Neurology Branch</div> | | |
| SECTION | | |
| INSTITUTE AND LOCATION <div style="text-align: center; font-weight: bold;">NINCDS, NIH, Bethesda, Maryland 20014</div> | | |
| TOTAL MANYEARS: <div style="text-align: center; font-weight: bold;">.25</div> | PROFESSIONAL: <div style="text-align: center; font-weight: bold;">.20</div> | OTHER: <div style="text-align: center; font-weight: bold;">.05</div> |
| SUMMARY OF WORK (200 words or less - underline keywords) <div style="padding: 10px;"> <p>A file has been created which can link Collaborative Perinatal Project data for women with relatives also in the Project. This has been imbedded in a <u>file</u> which classifies registrants by the number of Project pregnancies if they have no relatives registered. This larger file, has in turn, been merged with a file containing other <u>genetic</u> information, such as twin zygoty, and <u>medical and psychological data</u> for familial studies of malformations and other conditions.</p> </div> | | |

Project Description:

The objective of this study is to identify all relatives of graviorae registered in the Collaborative Perinatal Project and link their records to facilitate genetic studies of obstetric, pediatric, psychological and sensory data.

The main record linkage file and its auxiliaries have been completed. Although it is being preserved as a separate file, on one set of magnetic tapes, it is also being merged with medical and psychological data preliminary to actual use in genetic and family studies.

Publications: None

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| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER <div style="text-align: center; font-weight: bold;">Z01 NS 01515-10 DNB</div> |
| PERIOD COVERED <div style="text-align: center; font-weight: bold;">July 1, 1975 to June 30, 1976</div> | | |
| TITLE OF PROJECT (80 characters or less) <div style="text-align: center; font-weight: bold;">Rh Hemolytic Disease in Negro and White Infants</div> | | |
| NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT <div style="display: flex; justify-content: space-between; align-items: flex-start;"> <div style="width: 30%;">PI: A.F. Naylor</div> <div style="width: 35%; text-align: center;">Research Geneticist</div> <div style="width: 30%; text-align: right;">DNB NINCDS</div> </div> | | |
| COOPERATING UNITS (if any) <div style="text-align: center; font-weight: bold;">None</div> | | |
| LAB/BRANCH <div style="text-align: center; font-weight: bold;">Developmental Neurology Branch</div> | | |
| SECTION | | |
| INSTITUTE AND LOCATION <div style="text-align: center; font-weight: bold;">NINCDS, NIH, Bethesda, Maryland 20014</div> | | |
| TOTAL MANYEARS: <div style="text-align: center; font-weight: bold;">.05</div> | PROFESSIONAL: <div style="text-align: center; font-weight: bold;">.05</div> | OTHER: <div style="text-align: center; font-weight: bold;">.00</div> |
| SUMMARY OF WORK (200 words or less - underline keywords) <div style="text-align: center;"> <p>To carry out an investigation of a report in the literature that high <u>Rh</u> (but not <u>ABO</u>) antibody levels have smaller morbid effects in <u>black</u> than <u>white</u> babies, a data file under development, which is rich in information on both control and outcome variables, will be augmented with the needed laboratory test information.</p> </div> | | |

Project Description:

Objectives: To confirm a report that high Rh antibody levels have smaller morbid effects in Negro than in white babies, although this is not true for ABO antibodies.

Major findings: Preliminary and indirect confirmation has been obtained, from a small data sample under study, for reports in the literature that high Rh antibody titers are not as highly associated with serious morbidity in Negroes as in whites.

Proposed course: An intermediate data file being mainly created for use in familial studies of malformations and other conditions will be augmented with variables needed for this particular study.

Publications: None

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|---|---|--|------------------|-------------------------------|--|--------------------|---------------------|------------|----------------------|---------------------|------------|
| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER <div style="text-align: right; font-weight: bold;">Z01 NS 01754-08 DNB</div> | | | | | | | | | |
| PERIOD COVERED <div style="text-align: center;">July 1, 1975 to June 30, 1976</div> | | | | | | | | | | | |
| TITLE OF PROJECT (80 characters or less) <div style="text-align: center;">Growth and Intellectual Development of Children from Interracial Matings</div> | | | | | | | | | | | |
| NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT <table style="width: 100%; border: none;"> <tr> <td style="width: 40%;">PI: L. Willerman</td> <td style="width: 40%;">University of Texas at Austin</td> <td style="width: 20%;"></td> </tr> <tr> <td>OTHER: A.F. Naylor</td> <td>Research Geneticist</td> <td>DNB NINCDS</td> </tr> <tr> <td>N.C. Myrianthopoulos</td> <td>Research Geneticist</td> <td>DNB NINCDS</td> </tr> </table> | | | PI: L. Willerman | University of Texas at Austin | | OTHER: A.F. Naylor | Research Geneticist | DNB NINCDS | N.C. Myrianthopoulos | Research Geneticist | DNB NINCDS |
| PI: L. Willerman | University of Texas at Austin | | | | | | | | | | |
| OTHER: A.F. Naylor | Research Geneticist | DNB NINCDS | | | | | | | | | |
| N.C. Myrianthopoulos | Research Geneticist | DNB NINCDS | | | | | | | | | |
| COOPERATING UNITS (if any) <div style="text-align: center;">Department of Psychology, University of Texas at Austin</div> | | | | | | | | | | | |
| LAB/BRANCH <div style="text-align: center;">Developmental Neurology Branch</div> | | | | | | | | | | | |
| SECTION | | | | | | | | | | | |
| INSTITUTE AND LOCATION <div style="text-align: center;">NINCDS, NIH, Bethesda, Maryland 20014</div> | | | | | | | | | | | |
| TOTAL MANYEARS: <div style="text-align: right;">.05</div> | PROFESSIONAL: <div style="text-align: right;">.05</div> | OTHER: <div style="text-align: right;">.00</div> | | | | | | | | | |
| SUMMARY OF WORK (200 words or less - underline keywords) <p>A small subpopulation within Collaborative Perinatal Project children has been identified as being of mixed <u>black</u> and <u>white</u> parentage. Within the interracial group of matings there is no evidence that genetic or socio-economic differences are related to race of mother (or father). Thus socio-psychological influences, presumably operating through mother-child interactions, can be examined indirectly. Two papers have been published which indicate that, although early childhood differences are wholly negligible, children of white mothers eventually develop positive intellectual differentials. On the other hand, an analysis of <u>postnatal growth</u> now in manuscript suggests that early development of interracial children born to white mothers is slightly less favorable than for other interracial or monoracial children.</p> | | | | | | | | | | | |

Project Description:

Results of analyses of growth and intellectual development have been prepared for separate publication.

The conclusions reached in an initial publication in Science, that analyses of CPP interracial for IQ data indicate that race of mother as a postnatal environmental indicator accounts for much of the black-white IQ differences, have been strengthened in a paper published last year. Analyses of Bayley Mental and Motor Scores, taken at 8 months, when maternal social influences will have had little effect show no differences between children of black/white and white/black matings. Also a more convincing demonstration has been made of lack of bias in genetic or socio-economic factors affecting four year IQ on the paternal side.

The physical development data have been re-analyzed to compare all properly selected CPP interracial and monoracial matings. Hospital variation and other background factors were corrected for by multivariate regression. At birth children born to white mothers, whether by white or black fathers, are very similar in weight and length. Monoracial blacks are definitely smaller and interracials with black mothers may be intermediate in size (small numbers cloud the issue). At four months interracials with white mothers fall behind in weight (but catch up after one year), perhaps because of social stresses in the household. A paper has been submitted for publication and is in the process of revision.

Publications: None

PERIOD COVERED

July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

The Genetics of Intellectual and Motor Performance

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: Sarah H. Broman
Other: Paul L. NicholsResearch Psychologist
Research PsychologistDNB NINCDS
DNB NINCDS

COOPERATING UNITS (if any)

None

LAB/BRANCH

Developmental Neurology Branch

SECTION

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:

.04

PROFESSIONAL:

.04

OTHER:

.00

SUMMARY OF WORK (200 words or less - underline keywords)

An initial report has been published on genetics of infant mental test performance, in which scores of twins and singletons were compared. A study of the effects of social status and race on performance at four and seven years has also been published. Additional data for IQ and over 20 subtests at four and seven years are being analyzed in terms of familial resemblance, performance in twins, and group differences.

Project Description:

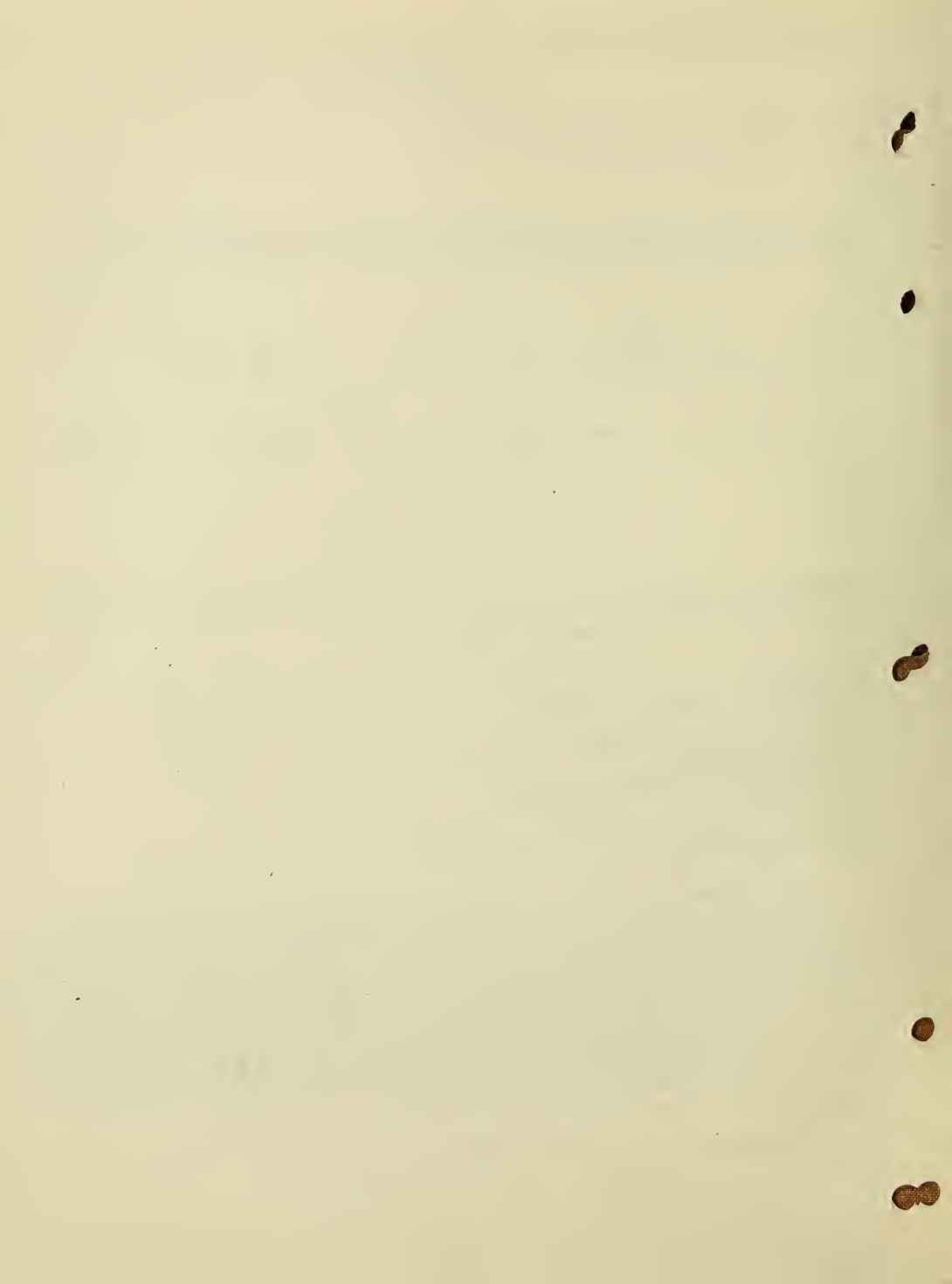
This study assessed the contribution of genetics to the variance in intellectual and motor performance at eight months, four years, seven years by correlating scores and measurements of twin, sibling, and half sibling pairs.

Publications:

Nichols, P. L. and Anderson, V. E.: Intellectual performance, race and socioeconomic status. Soc. Biol., 20: 367-374, 1973.

Nichols, P. L. and Broman, S. H.: Familial resemblance in infant mental development. Dev. Psychol., 10: 442-446, 1974.

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|--|---|---|-------------|-------------|-------|-------------|--------|---------------|-----------------|-------------|
| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 NS 02052-04 DNB | | | | | | | | |
| PERIOD COVERED July 1, 1975 through June 30, 1976 | | | | | | | | | | |
| TITLE OF PROJECT (80 characters or less) • The First Year of Life | | | | | | | | | | |
| NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT | | | | | | | | | | |
| <table style="width: 100%; border: none;"> <tr> <td style="width: 33%;">P.I.:</td> <td style="width: 33%;">J. S. Drage</td> <td style="width: 33%;">Chief</td> <td style="width: 33%;">DNB, NINCDS</td> </tr> <tr> <td>Other:</td> <td>E. C. Jackson</td> <td>Biostatistician</td> <td>OBE, NINCDS</td> </tr> </table> | | | P.I.: | J. S. Drage | Chief | DNB, NINCDS | Other: | E. C. Jackson | Biostatistician | OBE, NINCDS |
| P.I.: | J. S. Drage | Chief | DNB, NINCDS | | | | | | | |
| Other: | E. C. Jackson | Biostatistician | OBE, NINCDS | | | | | | | |
| COOPERATING UNITS (if any) J. B. Hardy, The Johns Hopkins University | | | | | | | | | | |
| LAB/BRANCH Developmental Neurology Branch | | | | | | | | | | |
| SECTION Perinatal Research Section | | | | | | | | | | |
| INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Md. 20014 | | | | | | | | | | |
| TOTAL MANYEARS: 0.2 | PROFESSIONAL: 0.1 | OTHER: 0.1 | | | | | | | | |
| SUMMARY OF WORK (200 words or less - underline keywords) | | | | | | | | | | |
| <p>"The First Year of Life" is planned as a volume to report on the frequency distribution of a number of findings reported on Collaborative Project children during the first year of their lives. It will include information on <u>birthweight-gestation distribution</u>, <u>bilirubin levels</u>, age at hospital discharge, and <u>distributions of various pathological findings</u> detected during the nursery stay and during the first year of life. Of particular interest will be information regarding <u>brain abnormality</u> as detected during the nursery period. This volume is intended to serve as a <u>general description</u> of the Collaborative Project children during their first year of life and as a reference document for further in-depth studies. The analyses for this work have been <u>completed</u>. The document will be ready for <u>publication</u> this year.</p> <p>See Contract Narrative N01-NS-2-2321.</p> | | | | | | | | | | |



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|--|---|---|-----------------|-----------------------|------------|--------------------|---------------------------|------------|
| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 NS 02058-04 DNB | | | | | | |
| PERIOD COVERED July 1, 1975 to June 30, 1976 | | | | | | | | |
| TITLE OF PROJECT (80 characters or less) Convulsive Disorders Data Analysis Group | | | | | | | | |
| NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT <table border="0"> <tr> <td>PI: K.B. Nelson</td> <td>Pediatric Neurologist</td> <td>DNB NINCDS</td> </tr> <tr> <td>PI: J.H. Ellenberg</td> <td>Mathematical Statistician</td> <td>OBE NINCDS</td> </tr> </table> | | | PI: K.B. Nelson | Pediatric Neurologist | DNB NINCDS | PI: J.H. Ellenberg | Mathematical Statistician | OBE NINCDS |
| PI: K.B. Nelson | Pediatric Neurologist | DNB NINCDS | | | | | | |
| PI: J.H. Ellenberg | Mathematical Statistician | OBE NINCDS | | | | | | |
| COOPERATING UNITS (if any) Dr. J. Freeman, Johns Hopkins Dr. K. Holden, Johns Hopkins | | | | | | | | |
| LAB/BRANCH Developmental Neurology Branch | | | | | | | | |
| SECTION | | | | | | | | |
| INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20014 | | | | | | | | |
| TOTAL MANYEARS: 0.9 | PROFESSIONAL: 0.6 | OTHER: 0.3 | | | | | | |
| SUMMARY OF WORK (200 words or less - underline keywords) <p>This study examines the relationship between perinatal factors and the occurrence of <u>seizure disorders</u> in childhood in a large, prospectively studied population. In addition to the central question of etiology, it investigates frequency, prognosis, demographic characteristics, and a number of other aspects of these disorders. Extensive hand review and classification of cases has been completed, and files created for this study. Univariate screen of maternal, obstetric, and pediatric risk factors, and demographic analysis, are in production runs. Multivariate analysis is planned. Selected topics of particular clinical relevance are under examination. Target date for completion of a manuscript for a monograph on childhood <u>epilepsies</u> is July, 1977.</p> | | | | | | | | |

Project Description:

Objectives: To examine maternal characteristics, conditions of pregnancy, labor, delivery and the neonatal period, and illness and injuries of early childhood for their association with seizure disorders. To seek clinically useful indices of prediction, to evaluate clustering of other handicaps with convulsive disorders, and to examine the frequency of seizure disorders in the population of the Collaborative Perinatal Project.

Methodology: The preliminary program to screen antecedent obstetric variables and early clinical manifestations with regard to their association with seizure disorder diagnoses has been completed. The analysis of demographic factors (e.g. institution, race, socioeconomic status, etc.) and their impact on the incidence and risk of seizure disorders is now available, and is in the process of assessment. An extensive study into the natural history of seizure disorders from one to seven years of life has been substantially completed, taking full advantage of the prospective nature of the Collaborative Perinatal Project.

Drs. Freeman and Holden are participating in the study of neonatal seizure states.

Major Findings: Approximately one in twenty children (57/1000) followed to the age of seven years had at least one seizure. About one-tenth that number (4.8/1000) had active epilepsy by the age of seven. As studied in the Collaborative Perinatal Project, active epilepsy in childhood is slightly more common in girls than boys, and approximately equal in rate in whites and blacks.

The most common seizure disorder in young children (or in any age group) is febrile seizures, found in almost 40/1000 of the Collaborative Project population. Febrile seizures are not considered to be epilepsy by most investigators, but the risk of epilepsy is increased among children who have had febrile seizures. Two percent of children with febrile seizures developed epilepsy by age seven in the Collaborative Project, and an additional 1% had at least one other seizure without fever. The risk of epilepsy was highest in children who were suspect or abnormal in neurological development before any seizure, and among those whose febrile seizure lasted more than 15 minutes, were multiple, or focal.

Seizures occurring in the first month of life were associated with a relatively high rate of death or subsequent disability, including cerebral palsy. Neonatal seizures occurred in 4.5/1000 of liveborn children in the Collaborative Perinatal Project.

Maternal, obstetrical, and early childhood characteristics which are associated with seizure disorders are now under study.

Future Course: Demographic data on convulsive disorders in Study children, and the univariate screen of antecedent maternal and pediatric characteristics are imminent. Multivariate analysis will follow. Selected topics of particular medical importance will be examined in sub-studies. A monograph on

convulsive disorders in childhood will be prepared; target date is July, 1977.

- Significance: The convulsive disorders are a common and socially costly medical problem. It is estimated that about 4 million Americans have some form of epilepsy. The cost of the epilepsies in direct payments and medical expenses has been calculated at more than \$4 billion per year. The information generated in the Collaborative Perinatal Project may significantly influence medical and social policy decisions having to do with this important problem area.

Publications: None

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|---|---|---|
| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 NS 02059-04 DNB |
| PERIOD COVERED July 1, 1975 to June 30, 1976 | | |
| TITLE OF PROJECT (80 characters or less) Cerebral Palsy Data Analysis Group | | |
| NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT | | |
| PI: K.B. Nelson PI: J.H. Ellenberg | Pediatric Neurologist Mathematical Statistician | DNB NINCDS OBE NINCDS |
| COOPERATING UNITS (if any) Dr. A. Leviton, Children's Medical Center, Boston, Mass. | | |
| LAB/BRANCH Developmental Neurology Branch | | |
| SECTION | | |
| INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20014 | | |
| TOTAL MANYEARS: 1.2 | PROFESSIONAL: 0.8 | OTHER: 0.4 |
| SUMMARY OF WORK (200 words or less - underline keywords) | | |
| <p>This large prospective study attempts to add to available knowledge of the perinatal factors associated with motor handicaps in childhood, the primary goal being to identify areas for possible preventive efforts.</p> <p>In the past year, data on demographic analysis and a univariate screen of maternal and pediatric factors associated with cerebral palsy have become available. Multivariate analysis is planned.</p> | | |

Project Description:

Objectives: To examine the etiology of motor disabilities in children, to improve clinical prediction, and to examine the relative frequencies of motor and associated disabilities in the population of the Collaborative Perinatal Project.

Methodology: The preliminary program to screen antecedent obstetric variables and early clinical manifestations with regard to their association with cerebral palsy diagnoses has been completed. The analysis of demographic factors (e.g. institution, race, socioeconomic status, etc.) and their impact on the incidence and risk of cerebral palsy is now available, and is in the process of assessment. An extensive study into the natural history of cerebral palsy from one to seven years of life has been substantially completed, taking full advantage of the prospective nature of the Collaborative Perinatal Project. Part of this material was presented at the American Academy for Cerebral Palsy in September, 1975, and will be submitted for publication. Dr. Alan Leviton (Children's Medical Center, Boston) is analyzing the data concerning acquired motor deficits.

Future Course: The multivariate analysis of the obstetric, early clinical and demographic factors is in the planning stage. Extensive use will be made of the NEUROMED statistical package developed in the Office of Biometry and Epidemiology to facilitate the rapid execution of this complicated last phase of analysis. Target date for completion of a manuscript for a monograph on cerebral palsy is July, 1977.

Major Findings: Cerebral palsy at seven years is somewhat more frequent in boys than girls, and among whites than blacks. Ten per cent of cerebral palsy is apparently caused by events occurring after the first month of life, most often infection or trauma.

Clearly handicapping cerebral palsy was present at age seven in 22-32/10,000 children, the range being related to race and sex. Within each birthweight and gestational age group examined, white males were at highest risk of cerebral palsy.

A listing of maternal and pediatric conditions most strongly associated with cerebral palsy outcomes has been made, and will be the basis for multivariate analysis.

Significance: Approximately 750,000 persons in the United States are victims of cerebral palsy; milder forms of cerebral palsy are more frequent still. As these individuals are afflicted from earliest childhood, the loss in social and economic terms is immense. Many are lifelong dependents of their families or the state. Our aims are to identify areas in which preventive efforts may be effectively directed, to improve clinical prognostication, to examine clustering of handicaps, and to estimate relative frequency of cerebral palsy conditions. Information in these areas may help to indicate fruitful directions for both medical and social policy decisions.

Publications: None

PERIOD COVERED

July 1, 1975 through June 30, 1976

TITLE OF PROJECT (80 characters or less)

Birthweight-Gestational Age

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

| | | | |
|--------|----------------|-----------------|------------------|
| PI: | B. H. Williams | Assistant Head | PRS, DNB, NINCDS |
| Other: | J. S. Drage | Chief | DNB, NINCDS |
| | K. D. McCabe | Consultant | DNB, NINCDS |
| | E. C. Jackson | Biostatistician | OBE, NINCDS |

COOPERATING UNITS (if any)

J. B. Hardy, The Johns Hopkins University
E. D. Mellits, The Johns Hopkins University

LAB/BRANCH

Developmental Neurology Branch

SECTION

Perinatal Research Section

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:

0.50

PROFESSIONAL:

0.25

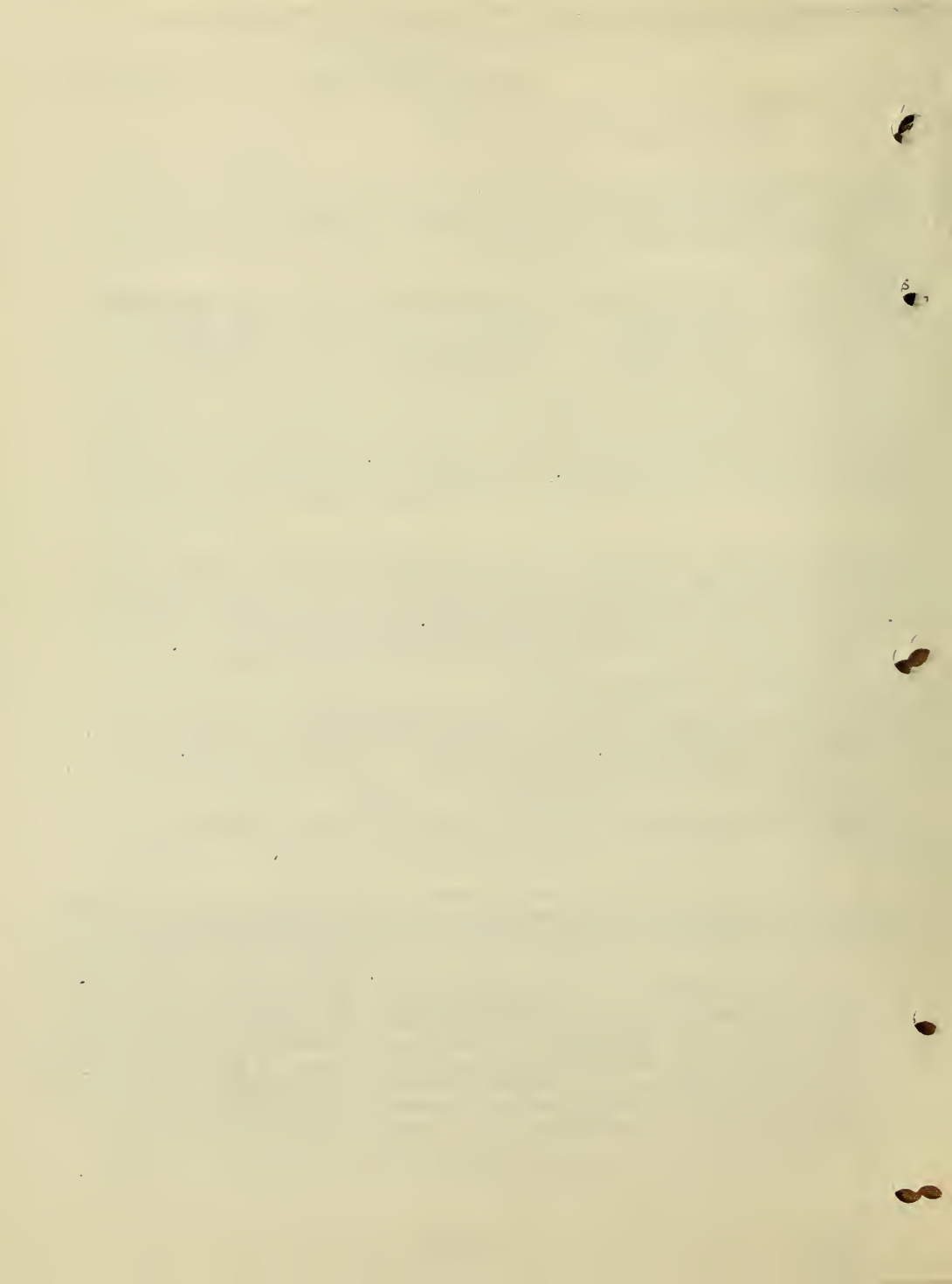
OTHER:

0.25

SUMMARY OF WORK (200 words or less - underline keywords)

During the past year the relationships of birthweight to placental weight, maternal education, socioeconomic index, gestation at registration, year of birth, institution, body length at birth and head circumference at birth were analyzed. The particular relationships of birthweight to placental weight and to gestational age with respect to survivors and non-survivors were analyzed through the application of a covariance program. The results of these studies are being compiled and constitute Phase I of the Birthweight-Gestational Age Study. A multivariate analysis is in progress to determine the relationship of birthweight as the dependent variable to a large number of antecedent (prenatal) independent variable. The component variable of a Birthweight Index have been established and will be explored to determine its predictive value for birth-weight-gestational age outcomes.

See Contract Narrative N01-NS-2-2321.



PERIOD COVERED

July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Minimal Brain Dysfunction

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: P. L. Nichols

Research Psychologist

DNB NINCDS

Other: Ta-chaun Chen

Sr. Math. Statistician

OBE NINCDS

COOPERATING UNITS (if any)

None

LAB/BRANCH

Developmental Neurology Branch

SECTION

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:

.75

PROFESSIONAL:

.65

OTHER:

.10

SUMMARY OF WORK (200 words or less - underline keywords)

A factor analysis of 25 possible signs of minimal brain dysfunction defined factors representing school achievement (learning disabilities), hyperkinetic-impulse disorders, social immaturity, and minor neurological problems (neurological soft signs). All children were given scores on each of the four factors, and correlation coefficients have been calculated between these scores and over 600 antecedent and concomitant variables. Many socioeconomic, maternal, neonatal, and developmental variables have significant associations with the factor scores. Children with symptoms in more than one area are more common than would be expected if there were no association among symptoms across the four areas, although most children who have problems in one area do not have problems in the other areas. Cases will be grouped by symptom patterns and examined separately for associations with antecedent variables. Twin and sibling studies suggest moderate genetic influence on the achievement factor score, low genetic influence on the hyperkinetic and immaturity scores, and still less influence on the neurological scores.

Project Description:

Specific diagnoses of minimal brain dysfunction have not been made for children in the longitudinal Collaborative Perinatal Project, but there is information available related to the most frequently cited symptoms (e.g., hyperactivity, learning difficulties, and equivocal neurological signs). This study will attempt to develop MBD criteria from the available CPP data, characterize MBD children in terms of demographic, psychological, and physical variables, and relate MBD or its components or individual symptoms to socioeconomic, maternal, neonatal, perinatal, and other antecedent variables.

Antecedent variables to be investigated will be selected from among family characteristics, maternal characteristics, the prenatal period, labor and delivery variables, the neonatal period, infancy and childhood characteristics, and intercurrent events.

Publications: None

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| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 NS 02106 - 03 DNB | | | | | | | | |
| PERIOD COVERED <p style="text-align: center;">July 1, 1975 to June 30, 1976</p> | | | | | | | | | | |
| TITLE OF PROJECT (80 characters or less) <p style="text-align: center;">Developmental Factors Associated with Mental Retardation</p> | | | | | | | | | | |
| NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT <table style="width: 100%; border: none;"> <tr> <td style="width: 33%;">PI:</td> <td style="width: 33%;">S. H. Broman</td> <td style="width: 33%;">Research Psychologist</td> <td style="width: 33%;">DNB NINCDS</td> </tr> <tr> <td>Other:</td> <td>P. L. Nichols</td> <td>Research Psychologist</td> <td>DNB NINCDS</td> </tr> </table> | | | PI: | S. H. Broman | Research Psychologist | DNB NINCDS | Other: | P. L. Nichols | Research Psychologist | DNB NINCDS |
| PI: | S. H. Broman | Research Psychologist | DNB NINCDS | | | | | | | |
| Other: | P. L. Nichols | Research Psychologist | DNB NINCDS | | | | | | | |
| COOPERATING UNITS (if any) <p style="text-align: center;">None</p> | | | | | | | | | | |
| LAB/BRANCH <p style="text-align: center;">Developmental Neurology Branch</p> | | | | | | | | | | |
| SECTION | | | | | | | | | | |
| INSTITUTE AND LOCATION <p style="text-align: center;">NINCDS, NIH, Bethesda, Maryland 20014</p> | | | | | | | | | | |
| TOTAL MANYEARS: | PROFESSIONAL: | OTHER: | | | | | | | | |
| .7 | .6 | .1 | | | | | | | | |
| SUMMARY OF WORK (200 words or less - underline keywords) <p> Data collected in the CPP are being analysed to determine primary and contributing roles of biological and environmental factors in mental retardation in a population of 40,000 children followed from the prenatal period to age 7. The incidence of severe retardation (0.5%) did not differ by ethnic group, but mild retardation was more frequent among blacks and Puerto Ricans (5%) than among whites (1%). Approximately 70% of the severely retarded, but less than 25% of the mildly retarded children had major neurological problems. <u>Perinatal anoxia</u> was associated with mental retardation at all ages, and <u>psychomotor test scores in infancy</u> were good predictors of school-age retardation. Mild retardation was associated with <u>lower socioeconomic status</u>. </p> | | | | | | | | | | |

Project Description:

Objectives: Data collected in the CPP are being analysed to determine the primary and contributing roles of biological and environmental factors in mental retardation in a population of 40,000 white, black and Puerto Rican children followed from the prenatal period to age 7. The identification of early signs of mental retardation will facilitate prevention, diagnosis and treatment, and will add substantially to knowledge in this area that has been largely derived from small retrospective studies of institutionalized retardates.

Method: Mental retardation was defined as an IQ of 70 or less on the Weschler Intelligence Scale for Children, or, for the relatively few children who could not be tested according to study protocol, equivalent IQs from other tests or reliable clinical judgements of retardation. Since children with IQs under 70 form a heterogeneous group, they were subdivided into four major categories consisting of those with severe retardation (IQ under 50) with and without signs of central nervous system damage, and those with mild retardation (IQ between 50 and 69) with and without such signs. Specific neurological diagnoses were obtained from the neurological examination given at age seven. The four groups were further subdivided by ethnic group and sex. Comparison groups are composed of children with IQs in the borderline, average and superior ranges.

Major Findings: Selected findings to date indicate that:

1. The incidence of severe mental retardation was approximately one-half of one percent in each of the ethnic groups studied (a total of more than 17,000 white, 19,000 Black and 1,000 Puerto Rican children).
2. The incidence of mild retardation was one percent among white children and almost five percent among Black and Puerto Rican children.
3. Approximately 70% of the severely retarded children in each of the three ethnic groups had major neurological problems.
4. Between 15 and 24% of the mildly retarded children had major neurological problems.
5. Signs of perinatal anoxia were associated with mental retardation at age 7 as well as at early ages.
6. Infant psychomotor test scores at 8 months of age were good predictors of mental retardation, especially the severe category, at age seven.
7. IQ scores at age four were also good predictors of mental

retardation as well as normal and superior intellectual performance at age seven.

8. Mild retardation, but not severe retardation, was associated with low socioeconomic status.

Proposed Course: Analyses of these data are continuing.

Publications: None

| | | | | | | | | |
|---|---|---|-----------------|-----------------------|-------------|----------------------|-----------------|-------------|
| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER <div style="text-align: right;">Z01 NS 02107-03 DNB</div> | | | | | | |
| PERIOD COVERED <div style="text-align: center;">July 1, 1975 through June 30, 1976</div> | | | | | | | | |
| TITLE OF PROJECT (80 characters or less) <div style="text-align: center;">The Study of Visual Abnormalities in the Collaborative Perinatal Project</div> | | | | | | | | |
| NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT <table style="width: 100%; border: none;"> <tr> <td style="width: 33%;">PI: R. Feinberg</td> <td style="width: 33%;">Research Psychologist</td> <td style="width: 33%;">DNB, NINCDS</td> </tr> <tr> <td>Other: E. C. Jackson</td> <td>Biostatistician</td> <td>OBE, NINCDS</td> </tr> </table> | | | PI: R. Feinberg | Research Psychologist | DNB, NINCDS | Other: E. C. Jackson | Biostatistician | OBE, NINCDS |
| PI: R. Feinberg | Research Psychologist | DNB, NINCDS | | | | | | |
| Other: E. C. Jackson | Biostatistician | OBE, NINCDS | | | | | | |
| COOPERATING UNITS (if any) W. R. Baldwin, Massachusetts College of Optometry; R. E. Hoover, Baltimore, Md.; R. P. Kling, Georgetown University Hospital; M. A. Whitcomb, Nat. Acad. of Sciences; S. Z. Wood, Washington, D. C., F. A. Young, Washington State University. | | | | | | | | |
| LAB/BRANCH <div style="text-align: center;">Developmental Neurology Branch</div> | | | | | | | | |
| SECTION | | | | | | | | |
| INSTITUTE AND LOCATION <div style="text-align: center;">NINCDS, NIH, Bethesda, Maryland 20014</div> | | | | | | | | |
| TOTAL MANYEARS: <div style="text-align: center;">1.2</div> | PROFESSIONAL: <div style="text-align: center;">1.0</div> | OTHER: <div style="text-align: center;">0.2</div> | | | | | | |
| SUMMARY OF WORK (200 words or less - underline keywords) <p> This project includes the analysis between visual abnormalities and outcome variables; anecdotal treatment based on case histories of unusual visual abnormalities; special studies of high-incidence disorders; and of concordance in siblings and twins; case studies of the blind children; and, preparation of a bibliography (monograph) encompassing these subjects. </p> | | | | | | | | |

Project Description:

The objectives of this project are to determine the extent to which genetic, maternal, obstetric, pediatric and environmental factors produce eye and visual abnormalities in the Study children; to assess the relative frequency of such anomalies; and, to study the concomitance of visual abnormalities with other sensory and motor neurological and systemic disorders.

A reference cohort of visual defects is defined for which the specified outcome will be tabulated. Outcome variables have been selected. Initial computer printouts have been obtained and are in process of being analyzed.

Publications: None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE
PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF
HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02108 - 03 DNB

PERIOD COVERED

July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Developmental Factors Associated with Learning Disorders

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER
PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: S. H. Broman
Other: P. L. Nichols

Research Psychologist
Research Psychologist

DNB NINCDS
DNB NINCDS

COOPERATING UNITS (if any)

None

LAB/BRANCH

Developmental Neurology Branch

SECTION

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:

.7

PROFESSIONAL:

.5

OTHER:

.2

SUMMARY OF WORK (200 words or less - underline keywords)

This study attempted to identify early behavioral, physical and familial characteristics of young children with a significant discrepancy between intellectual ability and school achievement. Low achievers, followed from the prenatal period to age 7, were compared with their IQ-matched academically successful controls on indices of cognitive and physical development and family characteristics. Wide-spread cognitive deficits and behavioral deviations were found in the preschool period. Indices of socioeconomic status and family structure were more strongly related to low achievement than were indices of physical development and medical status.

Project Description:

Objectives: Children with normal intelligence and poor school performance, particularly in reading, present significant problems in etiology and the development of effective remedial techniques. Longitudinal data collected in the CPP on a population of 40,000 children permit a study to be made of early events, beginning in the prenatal period, which differentiates between children with learning disorders in the first and second grades and those without a significant discrepancy between intellectual ability and school performance. The accurate identification of precursors of behavior patterns identified as learning disorders will facilitate prevention, early diagnosis and treatment. The objectives of this study are to determine the degree of association between learning disorders at age seven and the following classes of variables:

1) Biological factors

- a) Complications of pregnancy and delivery
- b) Adverse neonatal conditions
- c) Neurological and general medical status at one and seven years
- d) Childhood diseases and accidents
- e) Physical growth rates

2) Socio-environmental factors

- a) Socioeconomic status and social mobility of family
- b) Parental education
- c) Maternal intelligence level
- d) Family size and composition

3) Familial factors: occurrence of learning disorders in relatives

- a) Monozygotic and dizygotic twins
- b) Full and half-sibs

4) Cognitive and behavioral factors

- a) Mental and motor development and behavior ratings at eight months
- b) IQ scores, fine and gross motor development, concept formation and behavior ratings at age four
- c) Verbal and performance IQ scores, conceptual and visual-motor development and behavior ratings at age seven

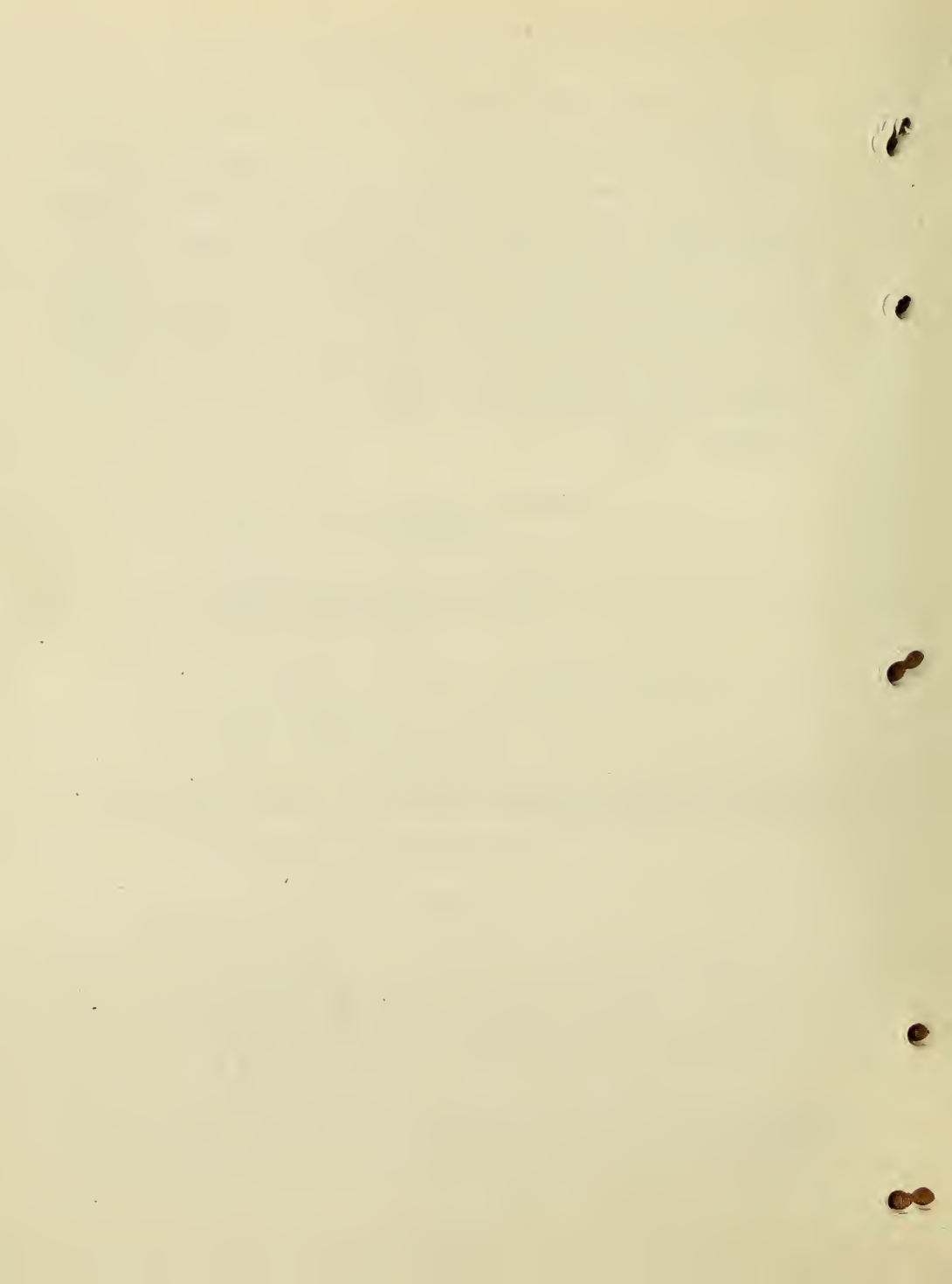
Method: A child was considered to have a learning disorder if he had an IQ of 90 or above on the Weschler Intelligence Scale for Children and was more than one year below grade expectancy (actual grade placement) in reading or spelling on the Wide Range Achievement Test. Children with

Learning disorders were compared with IQ-matched academically successful controls in order to isolate early signs and current characteristics. Comparisons were made within ethnic group (white, black and Puerto Rican).

Major Findings: (1) low achievers at age 7 showed fairly wide-spread cognitive deficits and behavioral deviations at age 4; (2) lower than expected school achievement at age 7 was accompanied by deficits in visual-motor performance, verbal ability and short-term memory, and by the presence of certain neurological soft signs; (3) indices of socioeconomic status and family structure were more strongly related to low achievement than were indices of physical development and medical status.

Proposed Course: Analyses of these data are continuing.

Publications: None



PERIOD COVERED

July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Comprehensive Analysis of the CPP Data on Congenital Malformations

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER
PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECTPI: N.C. Myrianthopoulos
OTHER: A.F. Naylor
D. RubinsteinResearch Geneticist
Research Geneticist
StatisticianDNB NINCDS
DNB NINCDS
OBE NINCDS

COOPERATING UNITS (if any)

C.S. Chung, Univ. of Hawaii; H. Lubs and M.L. Lubs, Univ. of Colorado; D. Smith,
Univ. of Washington; J. Frias, Univ. of Florida; M. Melnick, Univ. of Indiana

LAB/BRANCH

Developmental Neurology Branch

SECTION

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:

4.50

PROFESSIONAL:

3.70

OTHER:

0.80

SUMMARY OF WORK (200 words or less - underline keywords)

This is a long-term project to study the epidemiologic characteristics of congenital malformations in singletons and twins; to assess and interpret the influence of maternal, socioeconomic, neonatal, medical and other environmental factors on the occurrence of congenital malformations; to determine the risk of familial occurrence and to elucidate the role of genetic factors and the mode of inheritance of certain malformations; to determine the severity and clinical significance of congenital malformations and their associations with neurological, psychological and sensory handicaps; and to assess the long-range effects of malformations on survival, growth and development.

Project Description:

Objectives: This is a long-term project to study the epidemiologic characteristics of congenital malformations in singletons and twins; to assess and interpret the influence of maternal, socioeconomic, neonatal, medical and other environmental factors on the occurrence of congenital malformations; to determine the risk of familial occurrence and to elucidate the role of genetic factors and the mode of inheritance of certain malformations; to determine the severity and clinical significance of congenital malformations and their associations with neurological, psychological and sensory handicaps; and to assess the long-range effects of malformations on survival, growth and development.

Methodology: A congenital malformation is defined as a gross physical or anatomical developmental anomaly which was present at birth or was detected during the first year of life. Malformations have been classified into major and minor categories on the basis of their severity, threat to life and cosmetic significance.

The analysis is divided in 11 parts, and for each appropriate epidemiologic and statistical methods are being employed.

Current status and major findings:

I. Epidemiology of congenital malformations in singletons.

This part has been completed. About 15 percent of single-born children had one or more malformations. Half of the malformations were major. Only about a third of malformations observed during the first year of life were diagnosed at birth. Except for three minor malformations which were more frequent among Negroes, there were no significant differences in malformation incidences between Negroes and whites.

A report of this part has been published.

II. Epidemiology of congenital malformations in twins.

This part has been completed. Twins have significantly more major and minor malformations than singletons but the difference is wholly contributed by monozygotic (MZ) twins. The incidence of malformations in dizygotic (DZ) twins is the same as that in singletons. Monoamniotic twins have more malformations than diamniotic twins. Concordance rates are significantly higher among MZ than among DZ twins for all malformation categories but among specific malformations only those of the musculoskeletal system show significant differences.

A report of this part has been published.

III. A study of the effects of medical, genetic and socioeconomic factors in the occurrence of congenital malformations.

This part has been completed. Multiple birth, pregnancy complications

(mostly through hydramnios) and male birth were positively correlated with increased risk in major malformations, whereas maternal weight gain was negatively correlated with major malformations. Maternal diabetes during pregnancy was significantly correlated with single or multiple major malformations.

Over one-third of children of chronic alcoholic mothers show a constellation of developmental deficits and clinical symptoms known as the "fetal alcohol syndrome". An analysis of malformations in children of mothers who took dilantin during pregnancy shows that 11% of these children show a constellation of developmental deficits and clinical symptoms consistent with the "fetal hydantoin syndrome".

Reports of this part have been published.

IV. Special analysis of the effects of diabetes in the mother on the occurrence of congenital malformations in the offspring.

This part has been completed. The risk of having a malformed child in mothers with continuous diabetes is doubled compared to that of non-diabetic mothers, with regard to major malformations and increased significantly with regard to minor malformations. The effect seems to be associated with the severity of the disease and not with the intake of insulin.

A report for this part has been published.

V. Genetic studies of congenital malformations.

This part is in progress. The studies have been designed to derive empiric risk figures of repeating a malformation when it has once appeared in a family; to identify familial aggregations of specific malformations; and to clarify the mode of inheritance in identified familial aggregations.

The basic file to be used in the analysis has been completed. This file combines genetic information such as family linkages, twin zygosity, etc., with outcome variables like absence or presence of malformations, minimal brain dysfunction, mental retardation, speech and hearing disorders, seizures, cerebral palsy, and visual disorders. Work has been slow because errors were detected in several source files, including the Master Card File, requiring review-and-correction, and computer program changes.

A special genetic study of branchial arch anomalies has been initiated, in association with Dr. M. Melnick of the University of Indiana, and is now in progress.

VI. Study of associations of ABO and Rh blood types with congenital malformations.

This study has been designed to confirm earlier suggestions of association of blood groups with congenital malformations, based on small samples.

The study is now in progress. Several strong associations have been

detected and special analyses are being performed to identify random and no random associations.

A report is being written.

VII. Study of the clinical significance of minor malformations.

VIII. Longitudinal study of development, morbidity and survival of children with malformations.

These two studies have been designed in collaboration with Dr. David Smith of the University of Washington, Seattle, and Dr. C.S. Chung of the University of Hawaii. The objective of Part VII is to establish which minor malformations are worthwhile detecting and why, and which ones can be ignored or considered as normal variants. Part VIII is a continuation of Part VII and deals with the effects of single and multiple malformations on growth and development.

All cases with multiple malformations are now being reviewed. Preliminary analysis of the first 212 cases showed that 14% represent known malformations syndromes, 54% primary single malformations with accompanying secondary malformations, and 32% remain unclassified, probably a mixture of unknown syndromes and random associations.

The studies are now in progress.

IX. Study of the effects of maternal factors in the production of congenital malformations.

A pilot study has shown that maternal factors may play a role in the causation of some malformations. An extensive study utilizing all available malformation data through age 7 years is underway.

X. Study of the effects of major and minor malformations on outcome at 7 years.

This part will investigate the effects of major and minor malformations on neurological outcome, psychological test performance and speech, language and hearing performance.

XI. Correlation of minor chromosomal variants with congenital malformations.

This part is being carried out as a contract operation with Dr. Herbert Lubs, University of Colorado, Denver, as principal investigator. The study utilizes data which have been developed by five Collaborating Perinatal Project institutions which are currently participating in the Denver-based chromosomal study of minor variants. See Contract Narrative N01-NS-5-2326.

The study is now in progress.

Publications:

Myrianthopoulos, N.C.: Congenital malformations in twins: epidemiologic survey. Birth Defects, Orig. Art. Series Vol. XI, No. 8:1-39, 1975.

Chung, C.S. and Myrianthopoulos, N.C.: Factors affecting risks of congenital malformations. I. Analysis of epidemiologic factors in congenital malformations. Birth Defects, Orig. Art. Series Vol. XI, No. 10:1-22, 1975.

Chung, C.S. and Myrianthopoulos, N.C.: Factors affecting risks of congenital malformations. II. Effect of maternal diabetes on congenital malformations. Birth Defects, Orig. Art. Series Vol. XI, No. 10:23-38, 1975.

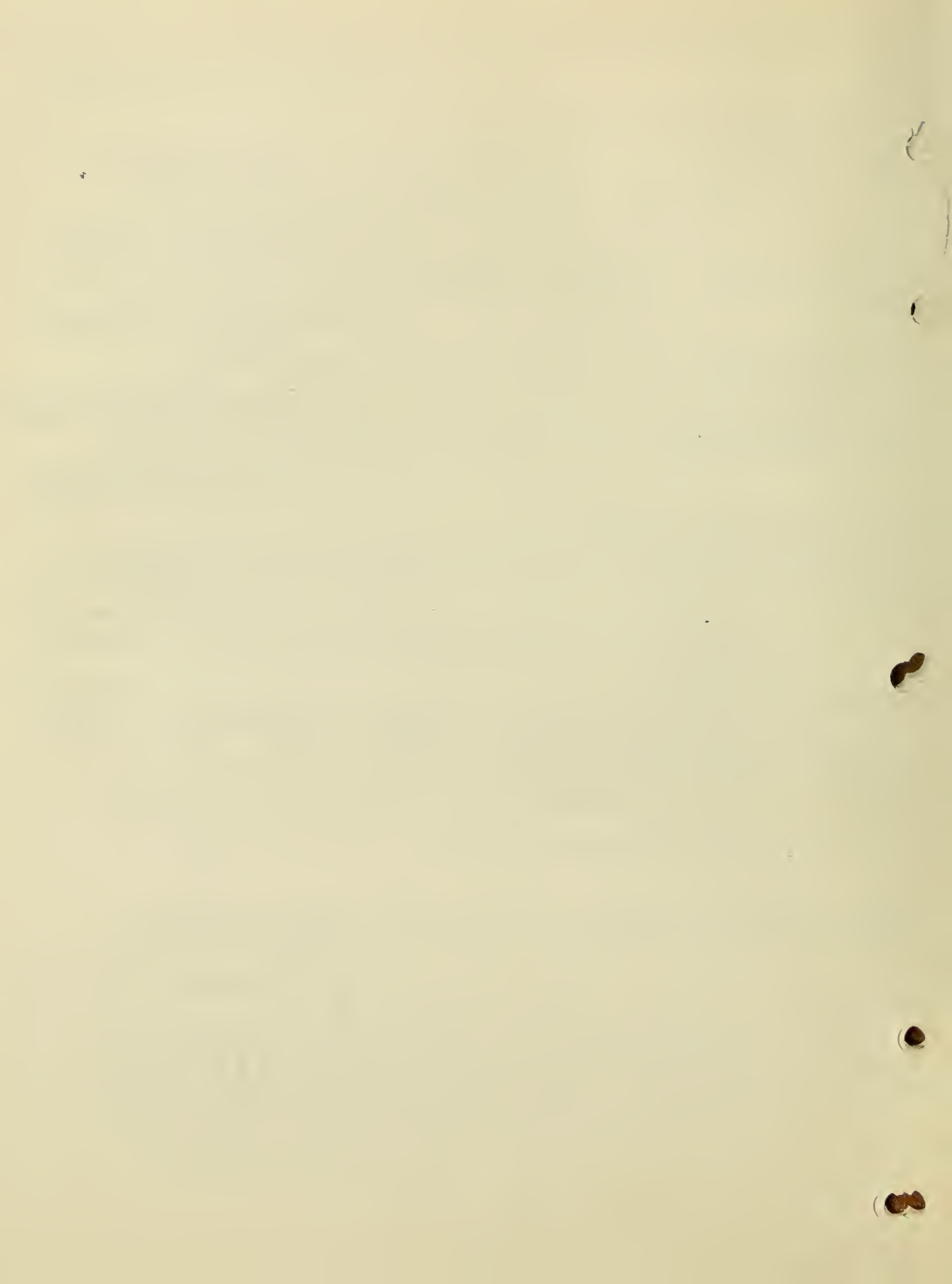
Smith, D.W., Klein, A.M., Henderson, J.R. and Myrianthopoulos, N.C.: Congenital hypothyroidism -- signs and symptoms in the newborn period. J. Pediat. 87:958-962, 1975.

Myrianthopoulos, N.C. and Burd , B.: A case of conjoined twins. Acta Genet. Med. Gemellol., in press.

Myrianthopoulos, N.C.: Congenital malformations in twins. Acta Genet. Med. Gemellol., in press.

Myrianthopoulos, N.C.: Concepts, definitions and classification of congenital and developmental malformations of the central nervous system. Handbook of Clinical Neurology, Vol. 26, in press.

Hanson, J.W., Myrianthopoulos, N.C., Harvey, M.A.S. and Smith, D.W.: Risks to the offspring of women treated with hydantoin anticonvulsants, with emphasis on the fetal hydantoin syndrome. J. Pediat., in press.



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| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 NS 02110 - 03 DNB |
|--|---|---|

PERIOD COVERED

July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Delayed Motor Development at One Year: Antecedents and Outcomes

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

| | | | |
|-----|--------------|-----------------------|------------|
| PI: | S. H. Broman | Research Psychologist | DNB NINCDS |
| PI: | K. B. Nelson | Pediatric Neurologist | DNB NINCDS |

COOPERATING UNITS (if any)

None

LAB/BRANCH

Developmental Neurology Branch

SECTION

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:

.01

PROFESSIONAL:

.01

OTHER:

.00

SUMMARY OF WORK (200 words or less - underline keywords)

A diagnosis of delayed motor development at one year made a significant independent contribution to IQ variance at age four, and was the best discriminator between retarded and normal children at that age. The purpose of this study is to define this condition more precisely in terms of specific development levels, and to examine some of its antecedents and longer-range outcomes at age seven. Factors investigated include maturity at birth, physical growth, presence of neurological and other medical diagnoses at one and at seven years, socioeconomic status and education of the parents, and IQ at age seven.

Project Description:

A diagnosis of delayed motor development at one year made a significant independent contribution to IQ variance at age four, and was the best discriminator between retarded and normal children at that age. The purpose of this study is to define this condition more precisely in terms of specific development levels, and to examine some of its antecedents and longer-range outcomes at age seven. Factors to be investigated include maturity at birth, physical growth, presence of neurological and other medical diagnoses at one and at seven years, socioeconomic status and education of the parents, and IQ at age seven.

Publications: None

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|---|---|---|-----------------|-------|-------------|----------------------|-----------------|-------------|
| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 NS 02112-03 DNB | | | | | | |
| PERIOD COVERED July 1, 1975 through June 30, 1976 | | | | | | | | |
| TITLE OF PROJECT (80 characters or less) Neonatal Hyperbilirubinemia | | | | | | | | |
| NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT <table style="width: 100%;"> <tr> <td style="width: 33%;">PI: J. S. Drage</td> <td style="width: 33%;">Chief</td> <td style="width: 33%;">DNB, NINCDS</td> </tr> <tr> <td>Other: E. C. Jackson</td> <td>Biostatistician</td> <td>OBE, NINCDS</td> </tr> </table> | | | PI: J. S. Drage | Chief | DNB, NINCDS | Other: E. C. Jackson | Biostatistician | OBE, NINCDS |
| PI: J. S. Drage | Chief | DNB, NINCDS | | | | | | |
| Other: E. C. Jackson | Biostatistician | OBE, NINCDS | | | | | | |
| COOPERATING UNITS (if any) J. B. Hardy, The Johns Hopkins University | | | | | | | | |
| LAB/BRANCH Developmental Neurology Branch | | | | | | | | |
| SECTION Perinatal Research Section | | | | | | | | |
| INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland, 20014 | | | | | | | | |
| TOTAL MANYEARS: 0.10 | PROFESSIONAL: 0.05 | OTHER: 0.05 | | | | | | |
| SUMMARY OF WORK (200 words or less - underline keywords) <p> The <u>neonatal hyperbilirubinemia</u> study has been designed to assess the relation- ship of <u>intermediate levels of serum bilirubin</u> on the subsequent neurological and mental development of Collaborative Perinatal Project children. There has been increasing concern that neonatal serum bilirubin levels between <u>10-20mg%</u> may be damaging to the central nervous system, not in the classical sense of 'kernicterus' associated with levels above 20 mg%, but rather damaging in more subtle yet clinically significant ways. Neonates have been studied in five birth- weight-gestational age categories, by three <u>socioeconomic classes</u>, for a variety of outcome measures, including <u>mental and motor assessments</u> at age 8 months of age, and spectrum of <u>neurological findings</u> of age one year which will include <u>motor performance, reflexes, tone, abnormal movements, eye findings</u> and the over- all neurological classification of normal, suspect or abnormal. The analysis of Phase I of this study has been completed. <u>Publication</u> will occur this year. The analysis of Phase II which includes data obtained at age <u>seven years</u> is in progress. </p> <p>See Contract Narrative N01-NS-2-2321.</p> | | | | | | | | |

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| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 NS 02169 - 02 DNB |
| PERIOD COVERED July 1, 1976 to June 30, 1976 | | |
| TITLE OF PROJECT (80 characters or less) Long Term, Differential Effects of Obstetrical Medication on Infants | | |
| NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT | | |
| PI: S. H. Broman Research Psychologist DNB NINCDS PI: Y. Brackbill Georgetown University Hospital | | |
| | | |
| COOPERATING UNITS (if any) Georgetown University Hospital | | |
| LAB/BRANCH Developmental Neurology Branch | | |
| SECTION | | |
| INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20014 | | |
| TOTAL MANYEARS: .08 | PROFESSIONAL: .07 | OTHER: .01 |
| SUMMARY OF WORK (200 words or less - underline keywords) <p> The two-fold purpose of this study was to determine how long-lasting are the effects of <u>obstetrical medication on infant psychophysiology</u>, and to determine how long they continue to affect functions differentially. It has been reported that both <u>anesthetics</u> and <u>analgesics</u> depress the quality of infant test performance and that the drug produced dysfunction is not short-lived. Items from examinations at 4, 8, and 12 months of age that reflect CNS integrity are being analysed. Preliminary results indicate many differences in infant status and performance among anesthetic-analgesic groups. </p> | | |

Project Description:

Objectives: The two-fold purpose of this study was to determine how long-lasting effects of obstetrical medication on infant psychophysiology, and to determine how long they continue to affect functions differentially. It has been reported that both anesthetics and analgesics depress the quality of infant test performance and that the drug produced dysfunction is not short-lived. Items from examinations at 4, 8, and 12 months of age that reflect CNS integrity are being analysed. Preliminary results indicate many differences in infant status and performance among anesthetic-analgesic groups.

Proposed Course: Analyses of these data are continuing.

Publications: None

| | | |
|---|---|---|
| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 NS 02170 - 02 DNB |
| PERIOD COVERED July 1, 1975 to June 30, 1976 | | |
| TITLE OF PROJECT (80 characters or less) Offspring of Schizophrenics: Developmental Factors Related to Intelligence | | |
| NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT | | |
| PI: R. O. Rieder PI: S. H. Broman | Psychiatrist Research Psychologist | LPP NIMH DNB NINCDS |
| COOPERATING UNITS (if any) National Institute of Mental Health | | |
| LAB/BRANCH Developmental Neurology Branch | | |
| SECTION | | |
| INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20014 | | |
| TOTAL MANYEARS: .2 | PROFESSIONAL: .1 | OTHER: .1 |
| SUMMARY OF WORK (200 words or less - underline keywords) The objectives of this study are: 1) to assess the hypothesis that <u>offspring of schizophrenics</u> are more susceptible to <u>perinatal complications</u> than are the <u>offspring of normals</u> ; and 2) to determine if the <u>offspring of schizophrenics</u> show a consistent pattern of abnormal development over time, extending from the neonatal period through childhood. Correlations between perinatal and early childhood events and later tests of <u>intelligence</u> in the offspring of <u>schizophrenics</u> group were compared with those in a large normal group. | | |

Project Description:

The objectives of this study are: 1) to assess the hypothesis that offspring of schizophrenics are more susceptible to perinatal complications than are the offspring of normals; and 2) to determine if the offspring of schizophrenics show a consistent pattern of abnormal development over time, extending from the neonatal period through childhood. Correlations between perinatal and early childhood events and later tests of intelligence in the offspring of schizophrenics group were compared with those in a large normal group. This study is completed.

Publications: None

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| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 NS 02171-02 DNB |
| PERIOD COVERED July 1, 1975 to June 30, 1976 | | |
| TITLE OF PROJECT (80 characters or less) Compendium of Heritable Disorders of the Nervous System | | |
| NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT <div style="display: flex; justify-content: space-between;"> PI: N.C. Myrianthopoulos Research Geneticist DNB NINCDS </div> | | |
| COOPERATING UNITS (if any) None | | |
| LAB/BRANCH Developmental Neurology Branch | | |
| SECTION | | |
| INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20014 | | |
| TOTAL MANYEARS: 0.10 | PROFESSIONAL: 0.05 | OTHER: 0.05 |
| SUMMARY OF WORK (200 words or less - underline keywords) The purpose is to prepare a comprehensive list of all known <u>heritable disorders</u> of the <u>nervous system</u> , including disorders and malformation syndromes which, though not primarily neurological, have neurological involvement. | | |

Project Description:

Objectives: To prepare a comprehensive list of all known heritable disorders of the nervous system, including disorders and malformation syndromes which, though not primarily neurological, have neurological involvement.

Methods employed: Sources for the compendium are published reports in the past and current literature containing convincing evidence of familial occurrence.

Current status: The available reports to date, yielded over 900 heritable neurological disorders. These have been classified into 23 nosological categories, including malformations, spinal atrophies, ataxias, demyelinating disorders, epilepsies, metabolic disorders, neuromuscular disorders, neoplasms and vascular disorders, mental retardation, syndromes, and others. A first draft of the list has been produced and is now under revision.

Publications: None

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| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 NS 02172-02 DNB |
| PERIOD COVERED July 1, 1975 to June 30, 1976 | | |
| TITLE OF PROJECT (80 characters or less) Children with Moderate or Severe Cerebral Palsy <u>and</u> Severe Mental Retardation | | |
| NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT | | |
| PI: K.B. Nelson PI: S.H. Broman | Pediatric Neurologist Research Psychologist | DNB NINCDS DNB NINCDS |
| COOPERATING UNITS (if any) None | | |
| LAB/BRANCH Developmental Neurology Branch | | |
| SECTION | | |
| INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20014 | | |
| TOTAL MANYEARS: 0.3 | PROFESSIONAL: 0.2 | OTHER: 0.1 |
| SUMMARY OF WORK (200 words or less - underline keywords) | | |
| <p> <u>Perinatal risk factors</u> were examined in a group of children with moderate or severe <u>cerebral palsy</u> and severe <u>mental retardation</u>. Of 179 characteristics considered, 31 distinguished the severely handicapped group from controls. Only one of these significant risk factors, <u>low birthweight</u> of prior liveborn child, could be recognized at the time gravida registered for prenatal care. One additional factor, low weight gain in the current <u>pregnancy</u>, differed in the course of pregnancy between these groups. Four factors of <u>labor and delivery</u> (arrested progress of labor, lowest fetal heart rate in second stage of labor, use of mid forceps, and low placental weight) distinguished affected children from controls. Twenty-five characteristics of the neonate were different, and most were markedly different, in children who were later severely handicapped as compared with controls. The greatest differences had to do with the occurrence of neonatal seizures, intracranial hemorrhage, neonatal neurological abnormality, <u>respiratory difficulty</u>, small size at birth, and low hemoglobin and hematocrit. </p> | | |

Project Description:

Objectives: The gravity of disability of children with both cerebral palsy and severe mental retardation is so great that none is likely to become an independent citizen. Many are institutionalized, at a cost of approximately \$7000 per year per child. Each child so handicapped represents both a human tragedy and a very substantial societal burden.

Methodology: From a population of 45,300 children, 90 children were found who had moderate or severe degrees of cerebral palsy and an IQ below 50 at seven years, or met these criteria at one year and died before seven. Comparisons were made with a control population of more than 34,000 children unselected for motor or mental characteristics, with respect to 179 variables relating to maternal and family characteristics, obstetrical factors, and neonatal findings.

Major Findings: The double disability of cerebral palsy and severe mental retardation was present in 1/590 liveborn children in the Collaborative Project. Of the ninety children with both CP and severe mental retardation, half were institutionalized by seven years. Seventy per cent survived at least to their seventh birthdays. Thirteen (14%) acquired their grave disabilities after the first month of life. Of factors significantly associated with serious mental and motor handicaps among the 50 children whose deficits were not "accounted for" by structural, metabolic, or other known factors, the most striking relationships were found among neonatal characteristics. In particular, four groups of factors appeared especially important: 1) small size at birth (low weight, length, head circumference), 2) difficulty initiating and maintaining independent respiration, 3) low hematocrit and hemoglobin, and 4) neonatal seizures. A discriminant function analysis indicates that the occurrence of neonatal seizures, intracranial hemorrhage, an overall impression of abnormal neurological status in the newborn nursery, 5 minute Apgar score, and the variables describing need for respiratory support, were the most effective independent discriminators between the severely handicapped group and controls.

Significance to Biomedical Research: It is a major concern of the Collaborative Perinatal Project to identify antecedents to childhood neurological disability, to suggest where preventive efforts may be directed with best effect. The present study suggests that, for the extremely disabled, neonatal abnormalities and certain observations during labor are much more strongly related to bad outcome than earlier predictors involving maternal and family characteristics, and that interventions focussing upon labor and neonatal periods may be most productive in attempting to prevent such very unfavorable outcomes.

Proposed Course: A manuscript describing the results of this study is near to completion. With its publication, this study will terminate.

Publications: None

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| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 NS 02234-01 DNB |
| PERIOD COVERED July 1, 1975 to June 30, 1976 | | |
| TITLE OF PROJECT (80 characters or less) Febrile seizures study | | |
| NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT <div style="display: flex; justify-content: space-between; margin-top: 20px;"> <div style="width: 30%;"> PI: K.B. Nelson PI: J.H. Ellenberg </div> <div style="width: 40%;"> Pediatric Neurologist Mathematical Statistician </div> <div style="width: 30%;"> DNB NINCDS OBE NINCDS </div> </div> | | |
| COOPERATING UNITS (if any) None | | |
| LAB/BRANCH Developmental Neurology Branch | | |
| SECTION | | |
| INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20014 | | |
| TOTAL MANYEARS: 0.6 | PROFESSIONAL: 0.4 | OTHER: 0.2 |
| SUMMARY OF WORK (200 words or less - underline keywords) <p>The aim of this effort is to evaluate the prevalence, associated conditions, and prognosis of <u>febrile seizures</u>, which is the most common <u>convulsive disorder</u> in any age group. Knowledge of the risks <u>associated with febrile seizures</u> is necessary for rational therapeutic decision-making. The Collaborative Perinatal Project presents a unique opportunity to evaluate, among other factors, the neurological status of children prior to the onset of any seizure, as well as other clinical characteristics, in a large, prospectively identified population.</p> <p>The initial report in this study has dealt with the likelihood of development of chronic <u>epilepsy</u> in children who have experienced febrile seizures. Work now in progress concerns the occurrence of later <u>mental retardation</u>, recurrence of febrile seizures, and <u>learning and behavior disorders</u> in this large group of children. Neurological and developmental status prior to the first febrile seizure is found to be an important determinant of outcome.</p> | | |

Project Description: Children in the population of the Collaborative Project who experienced febrile seizures were identified, and their seizure histories and subsequent histories examined.

Objectives: The aim of this effort is to evaluate the prevalence, associated conditions, and prognosis of this extremely common convulsive disorder. Knowledge of the risks associated with febrile seizures is necessary for rational decision-making as to therapy.

Methodology: Information abstracted from the records of children with febrile seizures was used to create a tape for data analysis on a time-share system in the Office of Biometry and Epidemiology. Clinical characteristics of children with febrile seizures, and other seizure experience by the age of seven years, as well as results of intelligence testing at seven years, were examined.

Results: Of 1821 children with febrile seizures in the Collaborative Project population, 1706 were followed to the age of seven years. Two per cent had become epileptic by the age of seven, and another one per cent had had at least one afebrile seizure not meeting the definition of epilepsy employed. Race, sex, birthweight and Apgar score were not significant predictors of epilepsy, but clinical features of the seizures and neurological status prior to the first seizure were of predictive value.

Other information on these children has been submitted to analysis, having to do with family history, mental retardation, etc., and these data are under-going analysis.

An invited paper on this subject was presented at the December, 1975 meeting of the American Epilepsy Society and at the American Epilepsy Foundation in January, 1976. A manuscript has been submitted for publication.

Significance: Four per cent of children experience at least one febrile seizure. This is the most common seizure disorder in any age group, and the most acute common problem in child neurology. Considerable disagreement as to optimal medical management exists, and it is our objective to supply clinically useful information as to the spectrum of risks associated with febrile seizures, as a component in therapeutic decision making.

Publications: None

ANNUAL REPORT
July 1, 1975--June 30, 1976

Epilepsy Branch
Neurological Disorders Program
National Institute of Neurological and Communicative Diseases and Stroke
National Institutes of Health

The Epilepsy Branch spent its first full year in the Federal Building following its relocation from Building 36, in January, 1975. All of the Branch operations are operating smoothly with the exception of the electronics and computer laboratories. Unfortunately, the environmental facilities which were planned for the Branch by NIH and the General Services Administration were not adequate because they did not meet our specifications. Heat and humidity controls are not adequate for video and audio tape stability. It has not been possible to utilize these laboratories to their full capacity for these reasons, and it is anticipated that during the next year, a satisfactory solution to the environmental problems may be found.

A drug, clonazepam (Clonopin), was marketed in August, 1975, for the treatment of absence seizures which have not responded to treatment with ethosuximide, and for the treatment of other nongeneralized seizures. Collaborative studies sponsored by NINCDS played an important role in providing data which was included in the new drug application and approved by the Food and Drug Administration. It is expected that a small but important contribution will be made by this new drug--the second marketed since 1960 as a primary drug in epilepsy. There are a number of potentially new drugs in investigational stages. Several of these are being evaluated by drug companies in animals, while others are in early human evaluation. Perhaps the most promising of these is valproate sodium (dipropylacetic acid; Depakene). This drug is widely used in 15 countries throughout the world, and now in clinical investigation in the United States. The Epilepsy Branch has evaluated this drug in pilot studies and will collaborate in a double-blind controlled trial in absence seizures at the University of Virginia. The drug was used in a similar pilot protocol in patients at the Clinical Center, NIH. Additionally, work in the pharmacology laboratory of the Branch has explored the mechanism of action of valproate.

The Epilepsy Branch has received national and international recognition for its contributions to research and the application of research findings to improving seizure control. By invitation, a number of lectures were given on intensive monitoring of patients with seizures, practical pharmacokinetics of antiepileptic drugs, new antiepileptic drugs, and the classification of seizures. The Branch received visitors from the pharmaceutical industry, from collaborators, the press, and from University faculty interested in exchange of information and learning about advances; more than 10 investigators from overseas visited to exchange international information on topics of mutual interest. At the Annual Meeting of the American Epilepsy Society in December 1975, J. Kiffin Penry, M.D., served as President of the organization. On the international level, he is Secretary-General of the International League Against Epilepsy. This organization is working towards amalgamation with the International Bureau of Epilepsy as a single voice--Epilepsy International.

While all of these activities are time demanding, they are valuable in augmenting a leadership role for epilepsy in the Institute, and help to carry Institute-initiated programs forward on an international and national basis.

Three comprehensive epilepsy programs were conducted under research contracts during the year. The programs funded are: The University of Virginia, Charlottesville; the University of Minnesota, Minneapolis; and Good Samaritan Hospital and Medical Center, Portland, Oregon. Contracts were awarded on a competitive basis to those who demonstrated evidence for the feasibility of establishing a program in their geographic area. Each also had plans to facilitate applied research to patients, and to coordinate research and teaching with health care services within a defined geographic area for persons with all types of epileptic seizures. Each program was required to have inpatient facilities available when required for periods from 3 to 6 months. Here special diagnostic evaluation and intensive treatment of seizure disorders is obtained. Vocational training, psychological support, other ancillary services, and continued schooling are also provided. Existing services to persons with epilepsy are coordinated by the program for maximum utilization--a key feature of the conduct of the comprehensive program in epilepsy. An integral part of the program is education for all levels of individuals in the management of patients with epilepsy: professionals, paraprofessionals and the laity. The Institute plans to fund 1 or 2 additional comprehensive epilepsy programs in this fiscal year.

A program for the initial pharmacologic evaluation of potential anticonvulsant drugs is in its second year, having begun January 1, 1975, at the University of Utah. The program receives compounds from medicinal chemists in academic and industrial settings at the rate of 70 to 80 per month. Primary activity evaluations are conducted in mice with the maximal electroshock and pentylenetetrazol anticonvulsant tests and the rotorod neurotoxicity test. Selected compounds received secondary evaluation. Twenty-five companies have signed agreements to participate in the program. A brochure describing the anticonvulsant screening project was developed during the year and mailed to 2500 potential suppliers to maintain interest and continue fresh input into the program. Compounds are received at NINCDS, and forwarded to the University of Utah for screening. Data on these compounds is returned to the Institute where chemical and biologic data are integrated into the Branch's minicomputer. Information about activity is supplied to the compound's supplier. A substantial investigation was made into the evaluation of marketed antiepileptic drugs. Sixteen drugs were evaluated in the test system to provide the basis for comparison of new compounds with proven drugs in the same test.

An important finding based upon collaborative research is that relatively low prophylactic doses of antiepileptic drugs do not significantly reduce the incidence of seizures which may follow head injury. At the University of Kansas Medical Center, a double-blind controlled study was conducted in 125 patients with 200 mg phenytoin and 64 mg of phenobarbital daily for 18 months, or placebo. While the study is not yet complete, indications are that the drugs employed did not reduce the risk of seizures when compared with the placebo group. This result compares with findings of another research contractor at the University of Washington. Prevention of seizures in the primate with focal lesion were studied. Although these posttraumatic seizures were

attenuated, they were not eliminated by prophylactic doses of the same drugs.

A pilot clinical research project concerned with complex partial seizures has been conducted since July 1, 1975, in the Clinical Center of the National Institutes of Health. Intensive monitoring of patients with this type of seizure is made through video recordings and telemetered electroencephalograms to document their clinical features. Patients with intractable seizures are intensively studied to attempt to improve their therapy. Results thus far have been encouraging, and plans are being discussed for continuation of this effort. Pilot pharmacokinetic studies are also possible in this setting.

The Branch has continued to support the monitoring of blood concentrations of antiepileptic drugs as a means of improving the treatment of patients with seizures. While it is not necessary to perform this laboratory examination in every instance, selected cases can benefit when toxic drug concentrations are reduced or insufficient concentrations are found. The latter problems may be due to poor patient compliance or difficulties in a patient's metabolism of the drug. The need for a medical epilepsy treatment service has been demonstrated in its first year. Although no attempt has been made to advertise its availability, more patient referrals are available than can possibly be accommodated.

A survey was conducted by the Epilepsy Foundation of America in cooperation with the Epilepsy Branch about the accuracy and uniformity of values reported by laboratories providing antiepileptic drug concentrations. The results of the survey were published: Pippen, CE; Penry, JK; White, BG; Daly, DD; Buddington, R: Interlaboratory variability in determination of plasma antiepileptic drug concentrations. Arch Neurol, 33:351-355, 1976. Wide interlaboratory variability was found; half of the laboratories reported results outside \pm one standard deviation of the mean of 5 reference laboratories. Because of laboratory variability, physicians often lack confidence in reported results. A voluntary quality control program among laboratories has been initiated to partially meet the need for greater quality control. Another aid will be certified antiepileptic drug standards in a biologic matrix to be available from the National Bureau of Standards. For the past two years, the Institute has supported interagency development of these products which are now undergoing field testing; certified standards will be available for purchase in the near future.

A significant number of children are affected by infantile spasms each year. Unfortunately, little is known about the etiology of this type of seizures and satisfactory treatment is lacking. To attempt to answer this need, the Institute has solicited research contract proposals for the quantification of infantile spasms, and a pilot study treatment with prednisone; another study will be addressed to the etiology of infantile spasms.

A book, Complex Partial Seizures and Their Treatment (Advances in Neurology, Volume 11, Raven Press) was published in December, 1975. The anatomical and pathophysiological basis for these seizures is described. Specific therapy, with emphasis upon the recently available carbamazepine is described.

The most recent data on bioavailability, metabolism, and blood concentrations of carbamazepine is discussed, as is the neurosurgical management and social aspects of this seizure type. Particular emphasis is given to the toxic aspects of carbamazepine and its use in the treatment of children.

The Epilepsy Branch is guided by the Epilepsy Advisory Committee and its Subcommittees. The Committee met twice in July, 1975, and May, 1976; The Subcommittee on Anticonvulsant Drugs, twice; Subcommittee on Basic Research, twice; and the Subcommittee on Epidemiology, once. The progress of NINCDS's sponsored research contracts and proposals for continued and additional studies is reviewed. Recommendations were made for program plans to be undertaken.

At least 4 United States pharmaceutical companies have investigational anti-epileptic drugs in various stages of development. It is encouraging to note the interest in this field, which may in part be due to this program's activities.

The program also continued its liaison activities with the Food and Drug Administration. J. Kiffin Penry, M.D., serves as a liaison member to the FDA's Advisory Committee on Neuropharmacologic Drugs. Three meetings were held during the year. Furthermore, response to a request from Bernard Cabana, Ph.D., consultation was provided regarding the bioavailability of phenytoin and suggestions made about the clinical investigation of this problem in patients with seizures.

In December, 1975, an international workshop was held on the classification of complex partial seizures. Scientists from the U.S., Germany and France, met in Bethesda to review their own and each others video tapes demonstrating particular aspects of the classification of complex partial seizures. Considerable progress was made at this meeting, and plans were made for further definition of this problem.

During the year, a review of the treatment of epilepsy with valproate sodium was published: Simon, D; Penry, JK: Sodium Di-N-propylacetate (DPA) in the Treatment of Epilepsy. *Epilepsia*, 16:549-573, 1975. Because of the international interest in this drug and awakening interest in the U.S., many requests for reprints of this article have been received.

A survey of 7 nursing homes in Montgomery County, Maryland, was made to investigate the use of the antiepileptic medications in their populations. The survey revealed 5.6% of the patients were receiving these drugs. Surprisingly, however, about 40% of the patients had no documented reason for the prescription (Mosely, JI; Penry, JK: Antiepileptic Medication in Chronic Care Facilities. *Public Health Reports* 90:140-143, 1975).

The Epilepsy Branch maintains a data base of 25,000 literature citations pertinent to epilepsy and related fields. These citations can be searched (at the request of staff, Institute members, or others in the scientific community) and retrieved from the DCRT computer files. Microfiche copies of each article cited are held within the branch. Currently, this data base is used as a valuable resource for the work of the Commission on Epilepsy and its Consequences. A major source of citations, Epilepsy Abstracts, is available to NIH on computer

tape as a part of a research contract with the Excerpta Medica Foundation. During the year, NINCDS and the National Library of Medicine entered into an agreement which made a complete file of these tapes accessible to all users of NLM's on-line data bases. This new data base ("File Epilepsy") was demonstrated at the meeting of the American Academy of Neurology, April, 1976, and has proven to be a popular source of information for neurologists. It was also used in the production of the Epilepsy Bibliography 1950-1975. This volume, containing 17,771 citations with author and key-word indexes, will be printed by the Government Printing Office and will be distributed to interested persons by NIH.

CONTRACT NARRATIVE
Neurological Disorders Program--Epilepsy Branch
July 1, 1975--June 30, 1976

NEW CASTLE STATE HOSPITAL (N01-NS-8-1310)

Title: Development of a Model for Assessment of New Anticonvulsant Agents

Contractor's Project Director: Joseph T. Brock, M.D.

Current Annual Level: \$91,052

Objectives: To further develop models to study the efficacy, safety and bioavailability of new antiepileptic drugs in humans. During the past year, investigations included study of the antiepileptic property of mexiletine as an adjunct in the therapy of patients with generalized seizures refractory to treatment, the evaluation of possible side effects of the drug, and evaluation of drug serum concentrations. A pilot study relating the dosage of another drug, sodium valproate, to serum concentration was performed.

Course of Contract: A study of mexiletine therapy and marketed drugs was completed toward the end of 1975. Clinical data was collected and sent to the Epilepsy Branch, NINCDS, Bethesda, for review and preparation for computer aided analysis. Analysis of that data is underway. During spring, 1976, trials were done with sodium valproate in 8 patients to correlate dosage to serum concentration.

Major Findings: Mexiletine would appear to be an effective anticonvulsant as therapy of seizures resistant to treatment. Additional data on short-term usage of mexiletine is being analyzed. A new study will determine the efficacy of valproate as an antiepileptic drug.

Significance to NINCDS Program and Biomedical Research: The pharmaceutical industry has demonstrated little interest in developing new antiepileptic agents. Aside from the economic factors involved, one of the industry's major problems is to obtain satisfactory clinical studies of antiepileptic drugs. Through this contract and others, NINCDS has supported clinical studies of antiepileptic drugs. Well controlled studies--as conducted at New Castle State Hospital--will be significant indicators of therapeutic merit of new antiepileptic drugs and may encourage the pharmaceutical industry to develop promising agents for clinical trial. It is anticipated NINCDS-sponsored studies will enable drugs to reach the market more readily, and thus be available to physicians who treat patients with seizures.

Proposed Course of Contract: Evaluations of other investigational antiepileptic drugs and further development of the model for drug evaluation are planned.

Publications:

Cereghino JJ, Brock JT, Van Meter JC, Penry JK, Smith LD, White BG: Carbamazepine for Epilepsy. A controlled prospective evaluation of multiple drug therapy. Clin Pharmacol Ther 18:733-741, 1975.

Cereghino JJ: Serum Carbamazepine Concentration and Clinical Control. In re Penry JK and Daly DD (Eds.), Complex Partial Seizures and Their Treatment. Raven Press, 1975, pp. 309-330.

Cereghino JJ, Wilder BJ, Kupferberg HJ, Yonekawa WD, Perchalski RJ, Ramsey RE, White BG, Smith LD, Penry JK: A single dose study of mexiletine (Ko 1173). Epilepsia 16:1975.

Cereghino JJ, Brock JT, Van Meter JC, Penry JK, Kupferberg HJ, Smith LD, White BG: A multiple dose study of mexiletine (Ko 1173). Epilepsia 16: 1975.

CONTRACT NARRATIVE
Neurological Disorders Program--Epilepsy Branch
July 1, 1975--June 30, 1976

UNIVERSITY OF VIRGINIA SCHOOL OF MEDICINE (N01-NS-9-2196)

Title: Absence Seizure Drug Studies

Contractor's Project Director: Fritz E. Dreifuss, M.D.

Current Annual Level: \$53,178

Objectives: To evaluate the effectiveness of investigational drugs on the frequency and intensity of absence (petit mal) seizures in patients previously untreated for this disease, and in patients who have failed to be controlled by ethosuximide; to evaluate drug effects on physiologic, psychometric, and other functions.

Course of Contract: Pilot studies in adults and children were completed to determine the effectiveness and safety of a new antiepileptic drug, valproate sodium (Depakene).

Major Findings: Dose-serum concentration relationships were determined and appropriate dose schedules devised. A protocol was developed for a double-blind controlled study of valproate sodium. Children with absence seizures will be treated with ethosuximide or valproate according to a proven data collection procedure developed by NINCDS--University of Virginia long term EEG telemetry and video recording used as an important measure of evaluating absence seizure frequency and duration.

Proposed Course: Valproate sodium, a promising investigational drug will be evaluated next fiscal year in a controlled study.

Publications:

Browne TR, Dreifuss FE, Dyken PR, Goode DJ, Penry JK, Porter RJ, White BG, White PT: Ethosuximide in the treatment of absence (petit mal) seizure. Neurol 25:515-524, 1975.

Penry JK, Porter RJ, Dreifuss FE: Simultaneous Recording of Absence Seizures with Video Tape and Electroencephalography. Brain 98:427-440, 1975.

CONTRACT NARRATIVE
Neurological Disorders Program--Epilepsy Branch
July 1, 1975--June 30, 1976

UNIVERSITY OF WASHINGTON (Nol-NS-0-2281)

Title: Complex Partial Seizure Drug Studies

Contractor's Project Director: Allan S. Troupin, M.D.

Current Annual Level: \$183,704

Objectives: To determine the relative antiepileptic efficacy and safety of carbamazepine and phenytoin in partial seizures; to measure drug concentrations in patients' blood; and to assess patients' psychological competence and social function. To conduct a pilot evaluation of clorazepate (Tranxene) as an adjunct to phenytoin therapy.

Course of Contract: Forty-seven patients completed the 10-month double-blind crossover study of carbamazepine. Eight patients were studied in the clorazepate study.

Major Findings: Carbamazepine was found to be a primary drug, as effective as phenytoin. The neuropsychological evaluation revealed improved high level concept formation and psychomotor problem solving with carbamazepine. These improvements are slight and probably not clinically significant. There were no clinically significant hematologic side effects observed with either drug.

Proposed Course: A double-blind evaluation with crossover will be made comparing clorazepate and phenobarbital as adjuncts to phenytoin treatment. Neuropsychological comparisons of the two drugs will be made. Drug serum concentrations will be correlated with drug dosage.

Publications:

Troupin AS, Green JR, Halpern LM: Tegretol as an anticonvulsant--a controlled double-blind comparison with Dilantin. Acta Neurol Scand Suppl 60:13-26, 1975.

Troupin AS, Ojemann LM: Paradoxical Intoxication--A complication of Anti-convulsant Administration. Epilepsia 16:753-758, 1975.

Dodrill CB, Troupin AS: Effects of repeated administrations of a comprehensive neuropsychological battery among chronic epileptics. J Nerv Ment Dis 161:185-190, 1975.

Dodrill CB: Diphenylhydantoin Serum Levels, Toxicity, and Neuropsychological Performance in Patients with Epilepsy. Epilepsia 16:593-600, 1975.

Dodrill CB: Effects of Sulthiame upon Intellectual, Neuropsychological and Social Functioning Abilities Among Adult Epileptics: Comparison with Diphenylhydantoin. Epilepsia 16:617-626, 1975.

CONTRACT NARRATIVE

Neurological Disorders Program--Epilepsy Branch

July 1, 1975--June 30, 1976

UNIVERSITY OF WASHINGTON (N01-NS-1-2282)

Title: Study of Experimental Antiepileptic Drugs in Animals

Contractor's Project Director: Joan S. Lockard, Ph.D.

Current Annual Level: \$236,000

Objectives: To compare the antiepileptic efficacy of drugs in spontaneous motor seizures of primates. Seizure frequency and behavioral toxicity are compared with drug dosage and drug blood concentration.

Course of Contract: This animal model of focal motor epilepsy has been utilized to determine the prophylactic efficacy of combined phenytoin/pheno-barbital treatment on posttraumatic epilepsy. Also, the basic kinetics of carbamazepine, valproate, and ethosuximide were studied in the monkey. The behavioral precipitance of seizures were studied in a group of free-roaming epileptic monkeys instrumented with motor seizure telemetry units.

Major Findings: A system for catheterization was developed whereby continuous administration of drugs and continuous monitoring of drug blood concentrations may be made. This system was used to determine the efficacy of valproate and clonazepam in this model. Six unrestrained animals with focal motor seizures were observed for 6 months using a fixed light/dark cycle in a free-room. Motor activity was used to telemeter EEGs; video recordings of seizures were made. This data forms a baseline for subsequent studies when drugs will be administered. A study of pharmacologic prophylaxis of post-traumatic epilepsy has been completed in this model. The prophylactically treated epileptic monkeys had partial motor rather than secondarily generalized tonic clonic seizures, considerably fewer seizures, lower EEG interictal spike counts, and a more consistent behavioral performance than placebo treated epileptic monkeys. The 4-month observation period following the prophylaxis study indicated that high doses of efficacious anticonvulsants may be required for lasting effects of drug therapy, and short periods of high drug doses may be preferred to longer period of lower drug doses. Also, in the short run, no-drug therapy may be better than low-drug dose therapy; earlier drug treatment may be more advantageous than later drug treatment; and drug withdrawal may have inherent problems with general health which accentuate epileptic activity. The final suggestion was that intensive pre-treatment with efficacious anticonvulsants was preferable to low prophylactic doses.

Proposed Course: The contract will be continued to further demonstrate and refine the usefulness of this animal model of epilepsy. Pharmacokinetics of new drugs are determined in the primate before efficacy experimentation is begun. Many other parameters are being followed: A 24-hour blood concentration of drug, sleep staging, continuous records of seizures, and the amount of interictal spiking. Studies in this model are underway to determine

if conditioning the animal will attenuate the occurrence of seizures. A 30-day intensive investigation of carbamazepine effectiveness is planned. Clonazepam and an investigational anticonvulsant will be evaluated.

Publications:

Lockard JS, Uhler V, DuCharme LL, Farquhar JA, Huntsman BJ: Efficacy of Standard Anticonvulsants in Monkey Model with Spontaneous Motor Seizures. *Epilepsia* 16:301-317, 1975.

Levy RH, Lockard JS, Green JR, Friel P, Martis L: Pharmacokinetics of Carbamazepine in Monkey Following Intravenous and Oral Administration. *J Pharm Sci* 64:302-307, 1975.

CONTRACT NARRATIVE
Neurological Disorders Program--Epilepsy Branch
July 1, 1975--June 30, 1976

UNIVERSITY OF KANSAS MEDICAL CENTER (N01-NS-2-2313)

Title: Investigation of Pharmacologic Posttraumatic Epilepsy Prophylaxis

Contractor's Project Director: Charles Brackett, M.D.

Current Annual Level: \$85,494

Objectives: A pilot study to determine the effectiveness of prophylactic treatment with diphenylhydantoin and phenobarbital in persons who suffer head injury and are thus liable to posttraumatic epilepsy.

Course of Contract: As of May 1, 1976, 125 patients have been accessioned in the study. Of this number, 104 patients have completed the required 18-month treatment period; they continue to be followed with neurological exams, plasma levels, and EEGs. There are 21 patients currently on drug therapy, and the study protocol is being scrupulously adhered to in their followup. Ten patients experienced seizures while on the study, and five have had seizures after completion of drug therapy. Data has been coded and stored in the PDP-11 mini-computer; analyses is underway.

Proposed Course of Contract: Analyses to answer the proposed hypotheses will be done, and further investigation will be made into the relationship between the study drug dosage and plasma levels which are somewhat lower than expected. Plans are being made based upon the pilot study results to continue to define the role of pharmacologic prophylaxis in posttraumatic epilepsy.

CONTRACT NARRATIVE
Neurological Disorders Program--Epilepsy Branch
July 1, 1975--June 30, 1976

EXCERPTA MEDICA FOUNDATION (N01-NS-3-2303)

Title: Publication of Epilepsy Abstracts, Volume 9

Current Annual Level: \$37,335

Objectives: To scan serial publications and periodicals from approximately 3000 of the world biomedical journals, select appropriate articles to be included in Epilepsy Abstracts in accordance with the guidance of the Project Officer and his editorial advisors; prepare abstracts with appropriate translations into English from foreign languages, classify, index, and store the abstracts in a computer retrievable form; and produce a 9-track computer tape for use at NIH. The Excerpta Medica Foundation produces camera-ready copy for each monthly issue of Epilepsy Abstracts, which includes an index of subjects and authors, and prints and distributes the journal monthly with a cumulative index at the end of the volume. In order to pay for the production of the camera-ready copy, the printing, and distribution, the Excerpta Medica Foundation sells subscriptions to recover the cost of production of camera-ready copy, printing, and distribution.

Course of Contract: Subscriptions to Epilepsy Abstracts, each at an annual cost of \$52.00, have been acquired from interested persons by Excerpta Medica at a satisfactory rate. Interest in the publication continues at a high level.

Proposed Course of Contract: Monthly issues have been distributed as scheduled; computer tapes have been delivered in accordance with the contract. This tape has been added to the Epilepsy Abstracts Retrieval System (EARS) data base, retrievable throughout the country via MEDLINE or TOXLINE terminals.

CONTRACT NARRATIVE
Neurological Disorders Program--Epilepsy Branch
July 1, 1975--June 30, 1976

ABBOTT LABORATORIES (N01-NS-3-2314)

Title: Synthesis and Testing of New Anticonvulsants

Contractor's Project Director: N. P. Plotnikoff, Ph.D.

Current Annual Level: \$59,383

Objectives: To synthesize a new series of benzopyran compounds and evaluate their potential anticonvulsant spectra in animals. The best candidate compounds will be evaluated for other pharmacologic properties.

Course of Contract: Approximately 160 compounds have been synthesized and evaluated for anticonvulsant activity in mice and rats. Several of these compounds are now undergoing final pharmacologic evaluation and appear promising.

Proposed Course of Contract: Further development of the most promising compound will be considered as findings are evaluated from clinical trials for indications other than epilepsy for two related drugs.

Publications:

Plotnikoff NP, Zaugg HE, Petersen AC, Arendsen DL, Anderson RF: New Benzopyrans: Anticonvulsant Activities. Life Science, 17:97-103, 1975.

CONTRACT NARRATIVE
Neurological Disorders Program--Epilepsy Branch
July 1, 1975 - June 30, 1976

STANFORD RESEARCH INSTITUTE (SRI) (N01-NS-3-2322)

Title: Development of a Wearable Eight-Channel EEG
Cassette Recording System

Contractor's Project Director: Charles S. Weaver, Ph.D.

Current Annual Level of Funding: \$146,606

Objectives: The objective of this effort is to design, fabricate, and clinically test prototypes of wearable EEG recording systems and associated playback systems which will make it possible to record eight-channels of EEG from ambulatory patients during a twelve-hour period using commercially available magnetic tape cassettes and to play these back at high speed for computer analysis and clinical evaluation. This development includes electrodes and preamplifiers to provide for eight-channels of differential recording connected to a wearable tape recorder with appropriate signal processing electronics. Digital encoding circuitry provides for a full dynamic range and suitable bandwidth to allow for automatic processing of EEG data by computer. In addition to the design and development of the recording technique and implementation in hardware, the overall system is to be clinically evaluated with a selected group of patients under environmental conditions similar to those in which it will ultimately be used. The playback system is to transcribe the encoded data from initial tape cassettes and reform that data onto conventional computer compatible digital tape, as well as to interface to a computer and provide real time analog outputs for strip chart recording.

Major Findings: A second generation four-channel prototype system was delivered in January 1975. The system weighs approximately two and one-half pounds, and records four-leads of EEG for twelve-hours on a standard C-120 audio cassette, with a bandwidth for each lead of 40Hz. The system is undergoing extensive evaluation at the Clinical Center, NIH, and elsewhere.

Significance to Biomedical Research and the Program of the Institute: The availability of a lightweight high-fidelity portable EEG tape recorder will allow significant improvement in both diagnosis and therapy of some types of epilepsy.

Proposed Course of the Contract: The development and construction of a recording and playback system that will record eight-leads of EEG waveforms together with the time of day will be completed.

Collaborating Units: This project is being carried out as a collaborative effort of the Neurological Disorders Program and the Fundamental Neurosciences Program. The Neurological Disorders Program is providing the clinical direction and the funding, while the Fundamental Neurosciences Program is providing the technical direction and project management.

CONTRACT NARRATIVE
Neurological Disorders Program--Epilepsy Branch
July 1, 1975--June 30, 1976

NATIONAL BUREAU OF STANDARDS (RFP-NINDS-74-02)

Title: Certified Antiepileptic Drug Reference Standards in Biologic Matrix

Contractor's Project Director: Robert Schaffer, Ph.D.

Current Annual Level: \$247,000

Objective: To develop standard reference materials for phenytoin, primidone, phenobarbital and ethosuximide in a biologic matrix.

Course of Contract: This project is an interagency agreement. Four anti-convulsant drugs were obtained from commercial sources and evaluated for purity. Various methods of analysis are being used, e.g., NMR, mass spectrometry, liquid chromatography, to purify the compounds. A stable biologic matrix has been developed.

Proposed Course of Contract: This project will end on June 30, 1976, when the standard reference materials incorporated in a plasma matrix will be ready for field evaluation. Cost of production of large batches of the product for field evaluation and stability studies will be borne by NBS, and the product marketed by that agency.

CONTRACT NARRATIVE
Neurological Disorders Program--Epilepsy Branch
July 1, 1975--June 30, 1976

UNIVERSITY OF CALIFORNIA AT LOS ANGELES (N01-NS-4-2330)

Title: Metabolic Studies of Eterobarb (DMMP)

Contractor's Project Director: Donald Jenden, M.D.

Current Annual Level: - 0 -

Objectives: To develop methodology for the analysis of eterobard (dimethoxymethyl phenobarbital; DMMP) and its metabolites and study the metabolism and pharmacokinetics of eterobarb in epileptic patients.

Course of Contract: The contract was initiated in June, 1974. Gas-chromatography mass-spectrometry methods had been adapted to the analysis of eterobarb and its metabolites to allow for the quantitation and elucidation of structure of small amounts of drug. Patient study has been completed.

Proposed Course: The study will be completed by June 30, 1976. A final report will be submitted to NINCDS summarizing all data.

Publications:

Goldberg MA, Gal J, Hodshown BJ, Cho AK, Jenden D: The Clinical Pharmacology of Eterobarb. Arch Neurol 33:393, 1976.

CONTRACT NARRATIVE
Neurological Disorders Program--Epilepsy Branch
July 1, 1975--June 30, 1976

UNIVERSITY OF UTAH (N01-NS-5-2302)

Title: Initial Pharmacologic Development of New Drugs

Contractor's Project Director: Ewart A. Swinyard, Ph.D.

Current Annual Level: \$129,000

Objective: To determine the anticonvulsant properties of novel organic compounds in mice at various times following intraperitoneal administration.

Course of Contract: Compounds are received by NINCDS from academic and industrial medicinal chemists and then sent to the University of Utah. The levels of anticonvulsant and neurotoxicity activities are determined. For those compounds which are found to be active, the ED₅₀ and TD₅₀ are determined. The pharmacologic data is then provided to NINCDS where it is reviewed and analyzed. The submitters of compounds are then informed of the results for their use for further synthesis. Compounds which are shown to be active will be selected for future development.

Proposed Course of Contract: This program began Jan. 1, 1975, and will continue for an additional 18 months during which time 1200 compounds will be screened for anticonvulsant activity. In addition, secondary screening techniques will be developed in order to help differentiate mechanism of action of the new agents.

CONTRACT NARRATIVE
Neurological Disorders Program--Epilepsy Branch
July 1, 1975--June 30, 1976

| <u>Contractor</u> | | <u>Project Director</u> | <u>Annual Level</u> |
|------------------------------|-----------------|-------------------------|---------------------|
| Univ of Minnesota | (N01-NS-5-2327) | R. Gumnit, M.D. | \$878,062 |
| Good Samaritan Hosp Portland | (N01-NS-5-2328) | J. Schimschock, M.D. | \$990,113 |
| Univ Va Med Center | (N01-NS-5-2329) | F. Dreifuss, M.D. | \$499,794 |

Title: A Comprehensive Epilepsy Program

Objectives: The objective of the Comprehensive Epilepsy Program is to facilitate applied research and to coordinate research and teaching with health care services related to persons with all types of epileptic seizures within a defined geographic area.

Course of Contract: Each contractor is conducting clinical and laboratory research in the diagnosis, treatment, prognosis and prevention of epilepsy. Each contractor is demonstrating to physicians and other professionals the newest advances in epilepsy research and treatment and is establishing a broad program for public education. In addition, each contractor is establishing the required procedures to assure, in a research setting, the availability to the person with epilepsy of complete and up-to-date preventive medical and rehabilitative psychological, vocational, educational, and social services.

Major Findings: During fiscal year 1974 each of the contractors performed a study to determine the feasibility of successfully establishing a Comprehensive Epilepsy Program in their area. The contractors showed evidence for the feasibility of establishing a program in their geographic area by a detailed description of clinical research capability, health care delivery, rehabilitation resources, etc., for the person with epilepsy.

Proposed Course: During the coming year, the Comprehensive Epilepsy Programs will facilitate applied research and coordinate research and teaching with health care services related to persons with all types of epileptic seizures within their defined geographic area. Inpatient facilities will be made available to patients for periods up to 3-6 months to receive special diagnostic evaluation and intensive treatment of their seizure disorders and any other concurrent handicap or physical problem. Vocational training, psychological support, other ancillary services and continued schooling will be simultaneously provided. Existing services will be coordinated for maximum utilization. An integral part of the program will be demonstration at all levels in the management of patients with epilepsy for professionals, paraprofessionals, and the lay public. While it is recognized that care for persons with epilepsy is available for many sources at the present time, these are scattered and noninclusive so that a patient may or may not receive total care depending on the local resources and how well they are coordinated. This program is incrementally funded on an annual basis.

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| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 NS 02097-05 EB |
| PERIOD COVERED July 1, 1975 to June 30, 1976 | | |
| TITLE OF PROJECT (80 characters or less) Diagnostic Value of Prolonged Telemetered EEG in Epilepsy | | |
| NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT | | |
| PI: J. K. Penry Others: S. Sato W. L. Brannon | Chief, Epilepsy Branch Visting Scientist Chief, Neurology Serv. | EB,NDP, NINCDS EB, NDP, NINCDS Naval Medical Center |
| COOPERATING UNITS (if any) U.S. Naval Medical Center, Bethesda, Maryland 20014 | | |
| LAB/BRANCH Epilepsy Branch SECTION | | |
| INSTITUTE AND LOCATION National Institute of Neurological and Communicative Disorders and Stroke, | | |
| TOTAL MANYEARS: 0.2 | PROFESSIONAL: 0.1 | OTHER: 0.1 Bethesda, MD |
| SUMMARY OF WORK (200 words or less - underline keywords) <p> Telemetered EEGs are recorded for 6 hours in order to sample over a longer period of time than the usual 20-30 minutes, and during normal activity. The incidence of diagnostic paroxysmal abnormalities in the 6-hour telemetered EEG are compared with those from routine conventional EEGs. About 10% of patients have had diagnostic abnormalities on the 6-hour telemetered EEG which were not recorded in the routine EEG. The accessioning of patients will continue. The ability to detect and record diagnostic epilepsy format abnormalities in the EEG after a single seizure will aid in the early treatment and long-term prognosis of patients who suffer their initial <u>seizure</u>. </p> | | |

Project Description:

Objectives: To develop a means of detecting and recording interictal paroxysmal abnormalities (epileptiform) in the EEG of patients who have suffered a clinical convulsion.

Methodology: Telemetered EEGs are recorded for 6 hours in order to sample over a period of time longer than the usual 20-30 minutes and during normal activity; in some patients, the latter may evoke interictal paroxysmal abnormalities. The study compares the incidence of diagnostic paroxysmal abnormalities in the 6-hour telemetered EEG with those from routine conventional EEGs.

Major Findings: In the initial group of patients studied, about 10% have had diagnostic abnormalities on the 6-hour telemetered EEG which were not recorded in the routine EEG. The accessioning of patients will continue.

Significance: If patients could be detected before the occurrence of a seizure, they could be treated and the seizure prevented, without treating those patients who will not have a recurrence.

Publication: None

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| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | | U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | | PROJECT NUMBER Z01 NS 02098--03 EB | |
| PERIOD COVERED July 1, 1975 to June 30, 1976 | | | | | |
| TITLE OF PROJECT (80 characters or less) Monaural Auditory Evoked Potential to Measure Anticonvulsant Effect on Brain Function | | | | | |
| NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT PI: J. R. Wolpaw Resident, Dept. of Neurol University of Vermont Other: J. K. Penry Chief, Epilepsy Branch EB, NDP, NINCDS | | | | | |
| COOPERATING UNITS (if any) Department of Neurology University of Vermont | | | | | |
| LAB/BRANCH Epilepsy | | | | | |
| SECTION -- | | | | | |
| INSTITUTE AND LOCATION National Institute of Neurological and Communicative Disorders and Stroke, Bethesda, MD | | | | | |
| TOTAL MANYEARS: 0.5 | | PROFESSIONAL: 0.3 | | OTHER: 0.2 | |
| SUMMARY OF WORK (200 words or less - underline keywords) The <u>monaural auditory evoked response</u> is used as a measure of acute and chronic anticonvulsant effect on central nervous system function. The origin and magnitude of the ipsilateral-contralateral peak latency difference were defined. Patients before and after initiation of anticonvulsant therapy are being evaluated. Results from a small number of patients allow no definite conclusions, but it appears that phenobarbital gives abnormally high ipsilateral-contralateral peak latency differences. | | | | | |

Project Description:

Objectives: To develop the ipsilateral-contralateral peak latency difference previously noted by others in the monaural auditory evoked response as a measure of acute and chronic anticonvulsant effect on central nervous system function.

Major Findings: Through an extensive study in normal controls the origin and magnitude of the previously reported ipsilateral-contralateral peak latency difference were defined. In pilot studies, moderate doses of ethanol and caffeine increased the ipsilateral-contralateral peak latency difference, often giving differences significantly higher than control values. At present, data is being collected from patients before and after initiation of anti-convulsant therapy. Results from patients allow no definite conclusions at this point, but it appears that phenobarbital gives abnormally high ipsilateral-contralateral peak latency differences.

Proposed Course: Continued collection of data from patients pre- and post-initiation of anticonvulsant therapy; continued follow-up of patients already in study; and continued ancillary control studies to further define the nature of the measurement and to arrive at the best possible method of measuring it.

Significance: This technique should provide the means of measurement of subtle, toxic effects of antiepileptic drugs on the central nervous system.

Publication: Wolfpaw JR, Penry JK: A temporal component of the auditory evoked response. Electroencephalogr Clin Neurophysiol 39:609-620, 1975.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE
PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF
HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02187-03 EB

PERIOD COVERED

July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Disposition of Mexiletine in Mice

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER
PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: R. J. Porter, Sr. Staff Associate
Others: H. J. Kupferberg Pharmacologist

EB, NDP, NINCDS
EB, NDP, NINCDS

COOPERATING UNITS (if any)

None

LAB/BRANCH

Epilepsy Branch

SECTION

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INSTITUTE AND LOCATION

NINCDS, NIH, Building 36

TOTAL MANYEARS:

0.2

PROFESSIONAL:

0.2

OTHER:

0

SUMMARY OF WORK (200 words or less - underline keywords)

Summary: Mice were given mexiletine [1-(2',6'dimethylphenoxy)-2-amino-propane] intraperitoneally and anticonvulsant activity determined by the maximal electroshock seizure test. Blood and brain levels of mexiletine were determined by gas-liquid chromatograph. Optically active d-benzl tartrate salts of mexiletine were made as a means of separating racemic mexiletine into its optically active antipods. The anticonvulsant activity of mexiletine in mice is of short duration, and its rate of disappearance from plasma parallels the anticonvulsant activity. Brain levels of mexiletine are much higher than plasma levels, due to its high lipid solubility. The ED₅₀ for mexiletine at 30 minutes following administration is 10 mg/kg and rises to 43 mg/kg at 3 hours.

Project Description:

Objective: To determine the blood and brain concentrations of racemic mexiletine and its antipods in mice in relation to its anticonvulsant activity.

Methodology: Mice were given mexiletine [1-(2',6'dimethylphenoxy)-2-amino-propane] intraperitoneally and anticonvulsant activity determined by the maximal electroshock seizure test. Blood and brain levels of mexiletine were determined by gas-liquid chromatography. Optically active d-benzyl tartrate salts of mexiletine were made as a means of separating racemic mexiletine into its optically active antipods.

Major Findings: The anticonvulsant activity of mexiletine in mice is of short duration, and its rate of disappearance from plasma parallels the anticonvulsant activity. Brain levels are much higher than plasma levels, due to its high lipid solubility. The ED₅₀ for mexiletine at 30 minutes following administration is 10 mg/kg and rises to 43 mg/kg at 3 hours.

Significance: Determination of the metabolic determination of mexiletine in mice provides the basis for clinical studies of this drug's disposition.

Publications: None

Project Description:

Objective: To study patients with complex partial seizures intensively.

Methodology: Patients are studied during three consecutive days. Intensive observation by a trained person, telemetered EEG, and 6 hours of video recording are done each day. During recording hours, the patient is continuously available for monitoring on the ward. Pre- and post-operative recordings of the patients are made. The telemetered EEG tapes are returned to the laboratory of the Epilepsy Branch where hard-copy tracings are made, and the video recordings are edited and the tapes reused.

Major Findings: Although the pilot study has not been completed, some observations are apparent: (1) The present video techniques are valuable in determining the qualitative nature of a patient's seizures, and are of some benefit clinically, especially for localization and determination of hysterical attacks; (2) Further investigation on differences in classification of seizure disorders is proceeding. An international workshop was held so that others with recorded data on complex partial seizures may share data and impressions. Recorded seizures from this pilot study were an integral part of this workshop; (3) It has been shown that surgical management of patients with epilepsy can be altered by the above techniques, although clear-cut guidelines have not yet emerged.

Significance: This pilot study has documented the usefulness of techniques of video and telemetered EEG recordings in the evaluation of complex partial seizures. It has provided a framework for a more extensive study of these techniques.

Proposed Course: This project will continue the study of additional patients.

Publications: None

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|--|---|--|-----------------------|------------------|----------------|-----------------|---------|----------------|----------------|-----------------|--|--------------|------------------------|-----------------------|
| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 NS 02188-02 EB | | | | | | | | | | | | |
| PERIOD COVERED July 1, 1975 to June 30, 1976 | | | | | | | | | | | | | | |
| TITLE OF PROJECT (80 characters or less) Identification of the Metabolic Pathway of Ethotoin in Humans. | | | | | | | | | | | | | | |
| NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT <table border="0" style="width: 100%;"> <tr> <td style="width: 15%;">PI:</td> <td style="width: 35%;">H. J. Kupferberg</td> <td style="width: 35%;">Pharmacologist</td> <td style="width: 15%;">EB, NDP, NINCDS</td> </tr> <tr> <td>Others:</td> <td>W. D. Yonekawa</td> <td>Pharmacologist</td> <td>EB, NDP, NINCDS</td> </tr> <tr> <td></td> <td>K. H. Dudley</td> <td>Asso. Prof. Pharmacol.</td> <td>Univ. of No. Carolina</td> </tr> </table> | | | PI: | H. J. Kupferberg | Pharmacologist | EB, NDP, NINCDS | Others: | W. D. Yonekawa | Pharmacologist | EB, NDP, NINCDS | | K. H. Dudley | Asso. Prof. Pharmacol. | Univ. of No. Carolina |
| PI: | H. J. Kupferberg | Pharmacologist | EB, NDP, NINCDS | | | | | | | | | | | |
| Others: | W. D. Yonekawa | Pharmacologist | EB, NDP, NINCDS | | | | | | | | | | | |
| | K. H. Dudley | Asso. Prof. Pharmacol. | Univ. of No. Carolina | | | | | | | | | | | |
| COOPERATING UNITS (if any) Department of Pharmacology University of North Carolina | | | | | | | | | | | | | | |
| LAB/BRANCH Epilepsy Branch | | | | | | | | | | | | | | |
| SECTION | | | | | | | | | | | | | | |
| INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20014 | | | | | | | | | | | | | | |
| TOTAL MANYEARS: 0.25 | PROFESSIONAL: 0.20 | OTHER: 0.05 | | | | | | | | | | | | |
| SUMMARY OF WORK (200 words or less - underline keywords) <p>Summary: Several hydroxylated metabolites of <u>ethotoin</u> were identified in urine of patients receiving ethotoin by gas-chromatograph mass spectroscopy. Five different <u>metabolites</u> were identified and quantitated. They are: ortho, meta and parathydroxyethotoin; 3,4,dihydroxyphenylethotoin; and 3 methoxy,4hydroxyphenylethotoin.</p> | | | | | | | | | | | | | | |

Project Description:

Objectives: To determine the metabolism of ethotoin in patients with seizures.

Major Findings: Five metabolites of ethotoin were identified and quantified. They are: ortho, meta and parathydroxyethotoin; 3,4,dihydroxyphenylethotoin; and 3methoxy,4hydroxyphenylethotoin.

Significance: Knowledge of the metabolism of this hydantoin provides the basis for comparison with other similar drugs available for the treatment of seizure disorders.

Proposed Course: This project has been completed.

Publication: Yonekawa W, Kupferberg HJ, Cantor F: A gas chromatographic method for the determination of ethotoin (3-ethyl-5-phenylhydantoin) in human plasma. In Schneider H (Ed): Clinical Pharmacology of Anti-epileptic Drugs, 1975, pp 115-123.

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| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 NS 01933 06 EB |
| PERIOD COVERED July 1, 1975 to June 30, 1976 | | |
| TITLE OF PROJECT (80 characters or less) Quantitation of Spike-wave Activity by a Reaction Time Method. | | |
| NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT | | |
| PI: J. K. Penry Others: S. Sato F. E. Dreifuss | Chief, Epilepsy Branch Visiting Scientist Prof. of Neurology | EB, NDP, NINCDS EB, NDP, NINCDS University of Virginia |
| COOPERATING UNITS (if any) Department of Neurology University of Virginia | | |
| LAB/BRANCH Epilepsy Branch | | |
| SECTION | | |
| INSTITUTE AND LOCATION National Institute of Neurological and Communicative Disorders and Stroke, Bethesda, MD | | |
| TOTAL MANYEARS: 0.2 | PROFESSIONAL: 0.1 | OTHER: 0.1 |
| SUMMARY OF WORK (200 words or less - underline keywords) | | |
| <p> This study determines whether reaction time in <u>absence</u> patients is or is not impaired in a gradual fashion from the point of spike-wave initiation. A <u>reaction-time</u> device is employed which gives instantaneous recognition by <u>voltage criteria</u> that a <u>spike-wave</u> burst has started. This burst is of much higher than normal background, and this factor alone is used to electronically trigger the reaction timer. On instantaneous recognition the reaction timer is triggered and a tone is delivered to the subject. The subject responds by turning off the high pitch tone with a telegraph key. Between paroxysms the patient is maintained in a state of alertness. </p> | | |

Project Description:

Objectives: To determine whether reaction time in patients with absence (petit mal) seizures is or is not impaired in a gradual fashion from the point of spike-wave initiation as has been suggested by some authors but disputed by others. There is some evidence for a "trough-like" pattern decrease of consciousness. The onset of decreased clinical functions during spike-wave paroxysms is evaluated by the reaction time method.

Methodology: A device is employed which gives instantaneous recognition by voltage criteria that a spike-wave burst has started. This burst is of much higher than normal background, and this factor alone is used to electronically trigger the reaction timer. On instantaneous recognition the reaction timer is triggered and a tone is delivered to the subject. The subject responds by turning off the high pitch tone with a telegraph key. Between paroxysms the patient is maintained in a state of alertness by a program of approximately 10 random stimuli per minute. All the data is collected by television, including a portion of the screen reserved for the reaction time from the digital clock. There is no age limit in selecting patients, but they must all have spike-wave paroxysmal discharge. A second group of patients was studied with the apparatus altered slightly so that the auditory stimulus was delivered 0.5 seconds into the seizure in order to see if responsiveness becomes less as the seizure progresses. Oscillographic displays from magnetic tape recordings of spike-wave paroxysms revealed shifting asymmetries.

Major Findings: The first group of patients suggest that some ability to respond early during the paroxysmal burst is maintained; this responsiveness is frequently not seen 1-2 seconds after onset. Analysis of responsiveness during short bursts suggests that patients may retain a normal reaction time during such paroxysms.

Significance: This study has applied video recording techniques and sophisticated electronic methods to improve the quality of clinical research. Specifically, this study is an analysis of the relation of the patient's behavior to his EEG during paroxysmal electroencephalographic events. An understanding of this relationship is important--not only as a guidepost for further research in the mechanism of epilepsy, but also in determining the day-to-day therapeutics of the epileptic patient.

Future Course: This project will continue. Patients from an investigational drug study will be evaluated.

Publications: None

| | | |
|--|---|--|
| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 NS 02235-01 EB |
|--|---|--|

PERIOD COVERED
July 1, 1975 to June 30, 1976

TITLE OF PROJECT (80 characters or less)

Metabolism of Methsuximide and Phensuximide in Epileptic Patients

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

| | | | |
|---------|------------------|-----------------------|---------------|
| PI: | H. J. Kupferberg | Pharmacologist | EB NDP NINCDS |
| Others: | W. Yonekawa | Pharmacologist | EB NDP NINCDS |
| | J. R. Lacy | Staff Associate | EB NDP NINCDS |
| | J. K. Penry | Chief, Epilpsy Branch | EB NDP NINCDS |

COOPERATING UNITS (if any)

None

LAB/BRANCH

Epilepsy Branch

SECTION

--

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:

0.25

PROFESSIONAL:

0.25

OTHER:

0

SUMMARY OF WORK (200 words or less - underline keywords)

The metabolism of the rates of N-dealkylation of methsuximide and phensuximide in epileptic patients were determined. Methsuximide 1200 mg and Phensuximide 1200 mg were administered to an epileptic patient at different times. The plasma levels of each drug and their N-dealkylated metabolites were determined by mass fragmentography. The t 1/2 of phensuximide following oral administration was 7 1/2 hours. The t 1/2 of the N-desmethyl phensuximide was similar to that of the parent compound. However, the plasma levels were only 1/3 that of phensuximide. The t 1/2 of methsuximide was 1 1/2 hours and that of the N-desmethylmethsuximide was 28 hours. It would appear from these results that the antiepileptic activity of methsuximide was due to N-desmethylmethsuximide whereas the activity of phensuximide was due to both parent and N-dealkylated metabolite.

Project Description:

Objectives: To determine the metabolic disposition of methsuximide and phensuximide in patients with epilepsy.

Major Findings: The metabolic half-life of the two drugs were determined as 7 1/2 hours for the former drug and 1 1/2 hours for the latter drug. The half-life for their metabolites was also determined.

Significance: Improved treatment of patients will be possible when the metabolism of these drugs is considered. For example, drugs with short half-lives should be administered more frequently than those with longer half-lives.

Proposed Course: Additional patients receiving these drugs will be investigated.

Publications: None

| | | |
|---|--|---|
| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 NS 02236-01 EB |
| PERIOD COVERED July 1, 1975 to June 30, 1976 | | |
| TITLE OF PROJECT (80 characters or less) Diagnostic and Therapeutic Reevaluation of Patients with Intractable Epilepsy | | |
| NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT | | |
| PI: Others: | J. K. Penry R. J. Porter J. R. Lacy | Chief, Epilepsy Branch, EB, NDP, NINCDS Sr. Staff Associate EB, NDP, NINCDS Staff Associate EB, NDP, NINCDS |
| | | |
| COOPERATING UNITS (if any) Television Engineering Section, CC, NIH | | |
| LAB/BRANCH Epilepsy Branch | | |
| SECTION | | |
| INSTITUTE AND LOCATION National Institute of Neurological and Communicative Disorders and Stroke, Bethesda, MD | | |
| TOTAL MANYEARS: 2.0 | PROFESSIONAL: 1.5 | OTHER: 0.5 |
| SUMMARY OF WORK (200 words or less - underline keywords) <p> Twenty patients with a long history of uncontrolled seizures had a complete examination, skull films, brain scan, EMI scan, and daily objective toxicity battery. Video recording and long-term telemetered EEGs established a seizure diagnosis in every patient. On admission, half of the patients exhibited medication toxicity, and half had poor seizure control. During hospitalization, phenytoin, phenobarbital, and primidone levels were determined daily. Carbamazepine, ethosuximide, and sodium valproate levels were measured less frequently. Efforts were made to obtain maximum therapeutic levels without toxicity. Of the patients who earlier exhibited medication toxicity, 3/4 became completely free of side effects. Most of these with poor seizure control on admission were improved.. Intensive monitoring techniques make possible better <u>diagnosis</u> and improved <u>treatment</u> of patients with intractable <u>epilepsy</u>. </p> | | |

Project Description:

Objectives: To study patients with intractable epilepsy and improve their seizure control.

Methodology: Patients are intensively monitored, using video recordings, telemetered EEGs, and serum drug concentrations. Specific seizure classification, when possible, provides the basis for improved therapy.

Major Findings: Improved seizure control and/or deduced drug toxicity was possible in more than half of the patients investigated.

Significance: Sophisticated evaluation of patients with intractable seizures yields benefits to the patient through research findings applied to clinical practice.

Proposed Course: Additional patients will be evaluated.

Publications: None

ANNUAL REPORT
July 1, 1975--June 30, 1976

Stroke and Trauma Program
National Institute of Neurological and Communicative Disorders and Stroke

I CEREBROVASCULAR DISEASE

Introduction

The stroke research program of the NINCDS is concerned primarily with studies of the control and disorders of cerebral circulation, the effect of changes in cerebral circulation on brain function, the pathophysiology of the cerebrovascular disorders, improved means for the prevention of stroke, better diagnosis of the type and site of stroke--particularly in its early phase, improved methods for the treatment of stroke and the biomedical consequences of stroke to the patient. The principal administrative instrument of stroke research development and support is the program of Stroke Clinical Research Centers. These 15 national stroke research centers are concerned with both the clinical research and the related fundamental research aspects of ischemic and hemorrhagic cerebrovascular disorders. Currently there are also 45 regular research grants. To reinforce the national stroke research effort, the NINCDS launched a pilot program of Stroke Acute Care Research Units (SACRU) to develop a network of acute stroke applied research units in the major hospitals throughout the country. Although not a primary responsibility of these programs, both the Stroke Clinical Research Centers and the Stroke Acute Care Research Units also serve as important resources for the training of investigators, medical students, residents and hospital medical staff, physicians in practice, nurses and other allied health personnel.

A. Pathogenesis

1. Cerebral Metabolism and Neurogenic Control of Cerebral Circulation

The brain requires an abundant and continuing energy supply in order to survive and function. Generation of this energy in the form of highly charged phosphate bonds, particularly ATP, is the principal purpose of cerebral oxidative metabolism and requires a constant blood borne supply of oxygen and glucose.

If energy production fails due to lack of substrate, enzyme systems (Na - K - ATP ase) that control the cellular ionic composition can no longer function properly causing K⁺ to leak out of the cells. These alterations in cellular permeability lead to development of intracellular edema.

With more severe ischemia, changes in protein and lipid metabolism occur leading to damage of cell membranes and impairment of cellular enzymatic processes which rapidly become irreversible. A combined approach to further the understanding of the effects of hypoxia-ischemia on the brain is being taken. This involves studies in

animal models of stroke and shock in which cerebral blood flow, regional glucose utilization, mitochondrial function, ion function, ion fluxes, redox state, tissue metabolites and ultrastructural studies are being examined. Clinical studies are being performed in patients with cerebrovascular disease in an attempt to prevent damage and to improve recovery.

Increasing emphasis is being given to the role of the autonomic nervous system in the control of cerebral circulation.

Patient Studies

Influence of adrenergic receptor blockade on circulatory and metabolic effects of disordered neurotransmitter function in stroke patients

Evidence for disordered cyclic AMP metabolism in patients with cerebral infarction

Prostaglandin levels in cerebral spinal fluid from patients following hemorrhage

Animal Studies

Effects of transient cerebral ischemia on neuronal viability and oxidative metabolism

Deoxyglucose method for the measurement of local glucose utilization

Measurements of brain redox state

The couple between cerebral metabolism and blood flow

Neurogenic factors in the cerebral blood flow response to chemical and pressor stimuli

Effects of cerebral anoxia on protein ribonucleic metabolism

In Vitro Study

Membrane protein phosphorylation

2. Role of Hypertension

The cause of essential hypertension remains obscure. While the search for an etiology in the past has focused on the pathogenic role of the kidney, of fluid and electrolyte imbalance and defective regulation of the aldosterone-renin angiotensin system, there has developed a new awareness that the CNS may also play an important role in the expression or even initiation of the disorder.

Studies

Experimental neurogenic hypertension

Study of the pathogenesis of Charcot-Bouchard aneurysms in hypertensive rabbits

Determination of risk factors of cerebrovascular disease in an urban hypertensive population

Animal studies on intracranial hypertension

Cooperative Study of stroke and hypertension

Primary intracerebral hemorrhage in the hypertensive--an autopsy study

3. Hematologic Aspects of Stroke

The blood platelet, the primary cell of hemostasis, has been put under recent scrutiny as an important contributor to thrombosis.

Although there is evidence suggesting that patients with cerebrovascular disease display abnormal or hyperactive platelet responses to physiologic stimuli, the data are limited. Availability of drugs which modulate platelet function makes possible more reliable clinical evaluations of platelet behavior in stroke patients.

Studies

Investigation of experimental platelet thrombi

Natural history study of stroke and relation to blood coagulation findings

Platelet function in stroke

Contribution of platelet aggregation and serotonin release to progressive cerebral infarction

4. Atherosclerosis

Studies

Morphological and experimental studies of the vascular wall including effects of ischemia and trauma on endothelium and endothelial alterations in arteries of miniature swine fed an atherogenic diet

Role of lipoproteins in cerebral atherosclerosis

B. Hemodynamics

Regional cerebral blood flow measurements continue to be explored to provide clinically useful information to confirm the diagnosis, to assess progress and to evaluate treatment in the stroke patient.

The techniques for measuring regional cerebral blood flow are well developed. These techniques reliably demonstrate areas of focal ischemia, permit measurement of changes in blood flow over a limited period of time and allow determination of the immediate effects of therapies such as induced hypertension, vasodilators, and anti-edema agents.

The introduction of an inhalation method now makes it feasible to perform serial measurements. Computer programs have been perfected to allow rapid on-line determinations of regional cerebral blood flow to be made so that this information can be used in the clinical management of patients. Therapies can thus be evaluated in terms of their effect on flow to the ischemic area as well as their clinical effect.

Patient Studies

Regional cerebral blood flow in man using the Obrist ¹³³Xenon inhalation method-comparison of the Xenon inhalation method and the Xenon injection technique

Regional cerebral blood flow and blood volume response to changes in cerebrospinal pressure in idiopathic dementia

Time course of change in regional cerebral blood flow following acute stroke

Measurement of cerebral blood volume in subarachnoid hemorrhage

Blood flow studies using the mass spectrometer

Isotopic measurement of regional cerebral blood flow before and after carotid endarterectomy

Animal Studies

Measurement of blood flow electrochemically has the advantage of providing regional measurements which can be monitored continuously.

In vivo method for determination of regional cerebral blood flow and regional oxygen utilization by intra-arterial injection of 15-oxygen tagged to water and oxyhemoglobin

C. Treatment

There is presently no proven method of treating patients with impending stroke or stroke in evolution and only conflicting experimental and clinical evidence on the efficacy of various forms of therapy.

Work with anticoagulants has failed to show any benefit for the patient with acute stroke except perhaps in the group suffering from recurrent emboli. With stroke in evolution the studies favor anticoagulation, but under most conditions there is little evidence that anticoagulants have altered the course of the disease for most patients.

Treatment with vasodilator agents such as Papaverine and Hexobendine have also been used, but the responses of regional blood flow have been variable. Slightly encouraging clinical results have been reported with the use of low molecular weight dextran in the treatment of acute stroke but the reasons for the clinical response or failure have not been demonstrated. A few dramatic clinical responses to induced hypertension have been reported, but again, the reason for this response in some patients and not in others has not been determined.

The variability in both clinical and experimental data makes therapeutic decisions difficult to make in treating stroke patients. It is evident that the pathological response of the brain to ischemic insult varies with the species studied. The ability to withstand total ischemia varies from 10 minutes in rabbits to 20 minutes in monkeys. In addition, the patterns of regional ischemic response vary from region to region with the same brain and from species to species.

It is suggested that approach to the therapy of cerebrovascular disease will have to be individualized to account for variations in age of infarct, site of occlusion, degree of collateral vessels, presence or absence of autoregulation, perfusion pressure, presence or absence of edema, blood viscosity, degree of tissue oxygenation, and other host factors as well.

The mechanism of recovery from stroke where flow is augmented is dependent more upon ultimate metabolic changes than blood flow itself. Studies have confirmed improved oxygen availability and utilization, and glucose metabolism under conditions where cerebral blood flow was increased to ischemic brain.

Patient Studies

Cooperative aneurysm study to test the efficacy of the following three forms of treatment in preventing rebleeding and reducing mortality during the first 14 days after entry of patient into the study:

1. Administration of an antifibrinolytic drug, either epsilon-aminocaproic acid or transexamic acid.
2. Antifibrinolytic drug therapy combined with a regimen of fluid restriction.
3. A regimen of fluid restriction and administration of an osmotic diuretic agent either glycerol or mannitol.

Study of the effect of betahistine (Serc^R) on recurrence rate of episodes of TIA

Sterotactic aneurysm thrombosis

Circulatory and metabolic effects of glycerol infusion in patients with recent cerebral infarction

Theophylline treatment in patients with large cerebral infarcts

Cooperative study of stroke and hypertension (452 patients)--antihypertensive therapy

Surgical treatment for aneurysm--attempts to improve surgical results by monitoring cortical oxygen availability

Animal Studies

Experimental cerebral infarction in the gerbil--effects of pharmacological agents

Examination of therapeutic effects of α adrenergic blocking drugs and dexamethasone in subarachnoid hemorrhage in primate model

Stroke Contracts

Bibliographic service on cerebrovascular diseases

Study of hospitals--frequency and character of transient ischemic attacks

Prognostic significance of initial symptoms of stroke

EMI feasibility studies (stroke)

TIA questionnaire evaluation

Oxidative metabolism and cerebral ischemia

Anatomy and physiology of cerebral neovascularization

D. Diagnosis

Conventional techniques do not record much information produced by the transmission of X-ray photons. Furthermore, once these have been recorded on radiographic film the eye is not able to discriminate between small differences in radiographic density. Since all soft tissues fall within a narrow band of approximately four percent variation in absorption coefficients, discrimination between different tissue densities within this band demand the use of highly sensitive detectors.

An apparatus has been devised which combines tomographic movements of a

narrow beam of conventional X-rays and sensitive photon detectors. The X-ray tube and the detectors scan the head linearly recording as many as 160 readings when the unit is rotated through one degree around the head and the process repeated. Ultimately when several thousand recordings have been made, they are processed in a computer.

This is an exciting medical breakthrough in scanning, since the system yields much more information than conventional X-ray systems and allows small variations in tissue density to be differentiated. The technique is completely non-invasive and the recordings are dramatic.

Patient Studies

Evaluation of computerized axial tomography in stroke

Development of non-traumatic screening methods using thermography, radionuclide angiography, and Doppler ultrasound

The relationship between radionuclide computerized tomography and X-ray computerized tomography in a group of patients with cerebrovascular disease

Reflex cutaneous plethysmography--a non-invasive technique for study of the cerebral circulation

Visual evoked potentials as a measure of cerebral dysfunction

Animal Studies

Microradiography of normal and hypertensive rabbits--accurate comparison of computerized axial tomograms with other radiological studies

Contrast materials and the blood brain barrier--cerebral angiography

Neurotoxicity of angiographic contrast agents

Acoustical detection of cerebral aneurysms and bruits

E. Epidemiology

Studies

Epidemiology of stroke--particularly the impact of TIA's and hypertension on the problem

Geographic differences in mortality from stroke in North Carolina

Epidemiology of stroke--Framingham population

Prospective study--mortality from cerebrovascular disease in groups of veterans in different geographic areas

Autopsy studies on atherosclerosis of the arterial circle of Willis in various ethnic groups

F. Natural History

Studies

Evaluation of 241 patients with transient ischemia and ischemic brain disease

Natural history of stroke and its relation to blood coagulation and findings

Immediate and long term prognostic indicators for the prevention and treatment of stroke

Correlation of original clinical findings including risk factors with regularly spaced follow-up studies on stroke patients--relationship of health resources (methods of treatment, rehabilitation, etc.) to survival of various types of stroke patients--determination of the socio-economic impact of stroke on the individual, his family and the community

G. Neuropsychology

Behavioral Studies

Aphasia research center--multidisciplinary research program on aphasia and related perceptual motor correlates of brain dysfunction

Early assessment, longitudinal monitoring and actuarial prediction of changes in the behavioral states of patients initially examined in the acute phase following onset of cerebrovascular symptomatology--correlation of neuropsychological test findings with clarifications yielded by other special diagnostic estimations of lesion size and location

Neuropsychological studies of cerebral symptomatology in stroke

H. Pediatrics

Studies

Stroke survey in childhood chronic subdural effusion in infancy

Neonatal anoxia-ischemia

I. Related Studies

Epilepsy in stroke

Central nervous system infarction and sickle cell hemoglobinopathy

Senile dementia

Migraine

Oral contraceptives and thromboembolism

J. Other

Cerebrovascular Workshop

Princeton Conferences

Survey report on cerebrovascular disease

SACRU

II SPINAL CORD INJURY

Introduction

Conventional thinking about spinal cord injury has been that spinal function is immediately and permanently lost following traumatic disruption of the cord. However, many years of laboratory studies with experimental spinal cord trauma conducted in universities and medical centers throughout the world now suggest that even severe physical injury does not always destroy the spinal cord. In some cases it has become evident that all of the tissue damage may not be directly related to the trauma, but may be an indirect result of it. Today, many scientists believe that the injury initiates a process of necrosis that eventually does destroy the cord. Within a definite time lag before this process is fully developed, the long fiber pathways are mostly intact. This fact raises the possibility that if this response of the tissue to injury could be stopped in the acute stage, then useful spinal cord function could be preserved and paralysis prevented.

To take advantage of new developments and to stimulate increased related research, the NINCDS is supporting 5 Acute Spinal Cord Injury Centers, 1 program project and 26 related research projects. Anatomical, physiological, and biochemical studies of normal and traumatized spinal cord offer the only possibility aside from prevention or regeneration of altering the disability occurring in 10-12,000 spinal cord injured patients each year.

A. Pathogenesis

Studies

Spinal cord biogenic amines in experimental spinal cord trauma-- Hypothesis is that the local tissue sequela of spinal cord injury are mediated by the excessive production and release of a catecholamine at the site of the injury. This catecholamine initiates changes in local vascular flow and permeability leading to necrosis.

Light and electronmicroscopic studies of pathologic changes in the spinal cord of animals sacrificed three months after injury

Investigations in animals and in man of pathophysiological alterations in urinary vesicle function resulting from spinal cord injuries

Investigation of the hypothesis that peroxidation of lipids (free radical reactions) is involved in the complex degenerative steps which follow traumatic spinal cord injury

Neurophysiological studies--The simplest way of studying neural conduction through the sensory tracts of the injured segment of the spinal cord is to record cerebral electrical potentials evoked by peripheral stimulation. The technique is essentially recording of an electroencephalogram during the repetitive stimulation of a peripheral sensory nerve.

Role of prostaglandins in spinal cord trauma

Spinal cord levels of a number of lysosomal and mitochondrial enzymes in spinal cord injury

Role of inflammatory factors following cord injury in animals

B. Treatment

The optimal treatment of spinal cord injury is not yet known. Experimentally several methods of treatment have been shown to be of some promise. These include local cooling of the injured spinal cord, parenteral administration of dexamethasone, myelotomy, and the use of drugs to prevent the increase of norepinephrine in the injured segment of the spinal cord.

Studies

Randomized treatment of patients in two categories; steroids and steroids plus epsilon aminocaproic acid--Patients are then divided into three categories for surgical treatment.

Pharmacological studies in animals with spinal cord injury--Effects of nitroglycerin alone and in combination with aramine (to maintain blood pressure)-- therapeutic agents are being sought which will:

1. Alter adrenergic nerve activity
2. Alter blood flow to the spinal cord
3. Stabilize membranes within the spinal cord
4. Prevent increase in prostaglandins after trauma

Clinical therapy--skeletal traction, early reduction of fracture dislocation, steroid medication and later spinal fusion

Animal study of the effects of myelotomy on normal and injured spinal cord

Spinal cord cooling in patients--use of agents which interfere with catecholamine synthesis

C. Diagnosis

Studies

Investigation of microvascular responses to spinal cord trauma by microangiographic techniques--effects of trauma on spinal cord circulation

Myelography in spinal cord trauma--improvement of radiographic techniques to determine the degree of compression of the spinal cord

Development of a computer-based system for evoked response testing in acute spinal cord injury--A non-invasive method for evaluation of cord and nervous system function which may serve as a useful predictor in the selection of treatment and evaluation of progress in a clinical setting.

D. Epidemiology and Natural History

Study

Determination of the significance of the initial intra-hospital care (including certain key aspects of emergency medical care) in influencing the mortality and morbidity of spinal cord injured patients--Study of the economic costs of spinal cord injury and provision of a data base for comparison between special spinal cord treatment centers and general hospitals. This includes direct costs, such as hospitalization, physical and vocational rehabilitation, professional services, as well as indirect costs, e.g., loss of income and projected loss of income.

III HEAD INJURY

Introduction

The main thrust of the head injury research supported by the NINCDS is directed toward a better understanding of the intracranial, pathophysiological, and metabolic consequences of head injury, the effect of head injury on systemic functions, and, in turn the cerebral complications of systemic dysfunction. Some progress toward these goals has been made in recent years largely because of the introduction of improved techniques for measurement of cerebral blood flow, cerebral metabolism of oxygen, and other substances. Systemic arterial pressure can now be continuously monitored and blood gases accurately measured. The CAT scan has made it possible to correlate morphological changes in the brain with pathophysiological and metabolic variables.

Earlier diagnosis and better general care has led to an improvement in the survival rate of patients with head injury. This care consists of close observation of the patient in a general or neurological intensive care unit, monitoring of vital signs, respiratory control and therapy, and maintenance of fluid and electrolyte balance and general body nutrition. The most important therapeutic agents are hypertonic solutions, hyperventilation, hypothermia, and steroids.

There have been two principal approaches to experimental head injury. The first is the study of models of mechanical brain injury ranging from acceleration and impact injuries of the head to the effects of pressure and various types of stresses on isolated nervous tissue. The second approach is the study of clinical head injuries in models that do not simulate head injury very well but permit the examination of a specific phenomenon in relative isolation from other complications of head injury. This approach encompasses investigation of experimental edema, ischemic and hypoxic hypoxia, and the effects of catecholamines and other "toxic" substances that might accumulate in the brain following head injury.

Clinical Studies

Regional cerebral blood flow studies using Xenon inhalation technique-- This non-invasive method allows for repeated measurement of regional cerebral blood flow and metabolism over a period of days. Changes in these variables are correlated with changes in the patient's neurological status and response to treatment.

Development of clinical mass spectrometry for the purpose of monitoring cerebral blood flow and oxygen consumption in brain injured patients on a continuing basis

Clinical pathogenesis of respiratory failure after head injury--determination of extra-vascular water in the lungs

Electrophysiologic correlates of acute brain insults--identification of the etiology of pressure waves and correlation of electrophysiological parameters (direct cortical response, steady potential, impedance, electrocorticogram) with the patient's neurological status and response to treatment

Ultrasonic visualization assessment of brain trauma--A diagnostic technique which provides information not available on angiography--possibility of scanning the brain much more frequently during the few critical hours immediately after injury than is possible by radiographic technique

Experimental Head Injury Studies

Methods have been developed for quantitative measurements of blood flow and oxygen consumption in rat cerebral cortex and whole brain. The metabolism of organic phosphates, glycolytic substrates, citric acid cycle intermediates and associated amino acids (in some instances also of neurotransmitters) has been measured during as well as following total ischemia

of 1-15 min duration, and in hypoglycemia. Cerebral blood flow and oxygen consumption, as well as tissue metabolites, have been measured in hypoxia, hypercapnia, hypocapnia, hypothermia and anesthesia. The results of these measurements have allowed a detailed description of circulatory and metabolic events, characteristic of each of these conditions.

An animal model more closely related to clinical head injury--development of a device which produces acceleration deceleration head injury in the monkey capable of creating a broad spectrum of physiological and pathological abnormalities which are predictable according to the force of the injury

Ultrastructural changes resulting from restoration of cerebral circulation following ischemia and from local freezing lesions

Contracts

Quantitative intracranial pressure measurement in man

Human responses to head injury

EMI feasibility studies (head injury)

Quantitative intracerebral pressure

IV REGENERATION IN THE CENTRAL NERVOUS SYSTEM

Regeneration of nerve cells in the severed mammalian spinal cord leading to a restitution of function has not been reproducibly demonstrated. Outgrowth of fibers has been shown to be abortive, presumably because of mechanical blockage due to glial or connective tissue proliferation. Recently there has been a revival of interest in this subject because of a number of advances in neurobiology. It has been shown that nerve fibers may grow continuously throughout life, or at least material synthesized in the cell body is constantly transported along the fiber. Recent electron microscopic investigation of synaptogenesis in the CNS indicates that these connections may be continuously "remodeled". It has also been noted that lesions in the CNS can stimulate extensive collateral sprouting of axons which potentially could establish functional connections. Successful regeneration of the severed spinal cord has been observed in the goldfish. Also, the extensive development of tissue culture methods for nerve cells, ganglia, spinal cord, and cortex has made available a variety of materials which might be implanted into the damaged spinal cord.

Much of the research supported by the NINCDS is concerned with the nature and extent of collateral sprouting that occurs after experimental lesions are made in the CNS. The results are extremely variable and probably reflect different growth potentials in different regions of the CNS.

As a result of recent developments in this field and the interest of the United States Congress in spinal cord injury, the Director of the NINCDS at

the request of the National Advisory Neurological and Communicative Disorders and Stroke (NANCDS) Council established an ad hoc Subcommittee on Growth and Regeneration in the Central Nervous System in October 1973. Its mission was to evaluate the current status of research on regeneration in the CNS and to identify those areas of research where potentially significant advances should be encouraged. A group of prominent basic scientists was appointed to the Subcommittee by the Director of the NINCDS, reflecting the very fundamental anatomical, physiological and neurochemical nature of the pertinent research. The mandate was accomplished by soliciting a number of manuscripts that provided relevant background information and data and by conducting four workshops. The first was a Workshop on Culture Techniques and Glial-Neuronal Interrelationships, under the chairmanship of Dr. Silvio Varon; the second, a Workshop on Neuronal Recognition and Synaptogenesis, under the chairmanship of Dr. Stanley Appel; the third, a Workshop on Structural and Functional Evidence of Plasticity in the Central Nervous System, under the chairmanship of Dr. Frederick Kerr; and the fourth, a Workshop on History of Science and Implication of Regeneration Research, under the chairmanship of Mr. Alan Reich. Summaries of this material and the recommendations of the Subcommittee on Growth and Regeneration in the Central Nervous System were presented to the NANCDS Council at its March 1975 meeting.

V MANIPULATION (e.g., Biomechanics; Chiropractic)

As part of the Senate Report on the Fiscal Year 1974 Appropriation for the NINCDS the Senate Appropriations Labor-HEW Subcommittee specified that.... "this would be an opportune time for an 'independent, unbiased' study of the fundamentals of the chiropractic profession. Such studies should be high among the priorities of the NINCDS...."

In order to provide the substantive scientific base necessary for this evaluation, the NINCDS convened a "Workshop on the Research Status of Spinal Manipulative Therapy." The Workshop was held at the NIH on February 2-4, 1975 and focused specifically on the documentation and evaluation of research results and clinical investigative experience. Spinal manipulative therapy was chosen as the theme of the Workshop since it is the primary therapeutic modality of chiropractic and would serve as a base for evaluating the scientific data about the fundamentals of chiropractic including the anatomy and pathophysiology of subluxation and methods of chiropractic diagnosis and therapy. The Workshop agenda included a detailed review of the history of manipulative therapy and discussion of the scientific issues of spinal geometry and kinematics, the intervertebral foramen, spinal root compression, spinal root and peripheral nerve pain, the pathophysiology of back pain, the concept of spinal vertebral subluxation, the clinical diagnosis of subluxation, and the evaluation of the efficacy of spinal manipulative therapy.

As a result of the discussions at the Workshop it was recommended that:

1. The NIH use its research grant program to stimulate and support additional basic and clinical investigations on spinal biomechanics and kinesiology, spinal reflexes, spinal root compression and

clinical investigations relevant to manipulative therapy.

2. Make staff and consultants available for technical assistance to institutions and organizations planning controlled trials of diagnosis and therapy.
3. Launch a program of predoctoral and postdoctoral research training of chiropractors and physicians for careers in research and clinical investigation relative to manipulative therapy.

RESEARCH OBJECTIVES OF THE STROKE AND TRAUMA PROGRAM FY 75

NUMBERS OF GRANTS AND CONTRACTS

CENTERS AND

| | PROGRAM PROJECTS | RESEARCH GRANTS | RESEARCH CONTRACTS | TOTALS |
|--|------------------|-----------------|--------------------|--------|
| 1. STROKE | 15 | 45 | 7 | 67 |
| EPIDEMIOLOGY AND NATURAL HISTORY | (15) | (1) | (2) | (18) |
| PATHOPHYSIOLOGY | (14) | (17) | (2) | (33) |
| DIAGNOSIS | (14) | (4) | (1) | (19) |
| TREATMENT | (13) | (20) | - | (33) |
| OTHER (CONFERENCES, SURVEYS, ETC.) | - | (3) | (2) | (5) |
| 2. CNS NEOPLASMS | - | 12 | - | 12 |
| 3. MANIPULATION (E.G. BIOMECHANICS; CHIROPRACTIC) | - | 1 | - | 1 |

RESEARCH OBJECTIVES OF THE STROKE AND TRAUMA PROGRAM FY 75

NUMBERS OF GRANTS AND CONTRACTS

CENTERS AND

PROGRAM PROJECTS RESEARCH GRANTS RESEARCH CONTRACTS TOTALS

| | | | | |
|----------------------------|-----|------|-----|------|
| 4. HEAD INJURY | 5 | 16 | 3 | 24 |
| PATHOPHYSIOLOGY | (5) | (12) | (2) | (19) |
| DIAGNOSIS | (5) | (1) | (1) | (7) |
| TREATMENT | (5) | - | - | (5) |
| OTHER (BASIC STUDIES) | - | (3) | - | (3) |
| 5. SPINAL CORD INJURY | 6 | 22 | - | 28 |
| PATHOPHYSIOLOGY | (6) | (7) | - | (13) |
| DIAGNOSIS | (5) | - | - | (5) |
| TREATMENT | (5) | - | - | (5) |
| OTHER (BASIC STUDIES) | - | (15) | - | (15) |
| 6. REGENERATION | - | 27 | - | 27 |
| 7. PERIPHERAL NERVE INJURY | - | 7 | - | 7 |

RESEARCH SUPPORT: NINDS STROKE AND TRAUMA PROGRAM

(DOLLARS IN THOUSANDS)

FISCAL YEAR 1975 (ACTUAL)

FISCAL YEAR 1976 (PROJECTED)

| | REGULAR GRANTS | | PROGRAM PROJECTS | | CENTERS | | CONTRACTS | | REGULAR GRANTS | | PROGRAM PROJECTS | | CENTERS | | CONTRACTS | |
|-----------------------------|----------------|-------------|------------------|-------------|-------------|-------------|-------------|-------------|----------------|-------------|------------------|-------------|-------------|-------------|-------------|-------------|
| | No. DOLLARS | No. DOLLARS | No. DOLLARS | No. DOLLARS | No. DOLLARS | No. DOLLARS | No. DOLLARS | No. DOLLARS | No. DOLLARS | No. DOLLARS | No. DOLLARS | No. DOLLARS | No. DOLLARS | No. DOLLARS | No. DOLLARS | No. DOLLARS |
| STROKE | 45* | 2393 | | | 15 | 5161 | 7 | 532 | 37 | 1907 | | | 13 | 5157 | 9 | 573 |
| HEAD INJURY | 16 | 639 | | | 5 | 1038 | 3 | 355 | 15 | 696 | | | 5 | 1479 | 3 | 328 |
| SPINAL CORD INJURY | 22 | 971 | 1 | 266 | 5 | 2076 | | | 18 | 822 | 1 | 271 | 5 | 2140 | | |
| GROWTH & REGENERATION | 27 | 1304 | | | | | | | 34 | 1905 | 1 | 315 | | | | |
| OTHER TRAUMA & INJURY | 7 | 291 | | | | | | | 7 | 237 | | | | | | |
| TUMORS | 12 | 616 | | | | | | | 14 | 724 | | | | | | |
| MANIPULATION (CHIROPRACTIC) | 1 | | | | | | | | 2 | 123 | 1 | 167 | | | | |
| TOTAL | 130 | 6214 | 1 | 266 | 25 | 8275 | 10 | 887 | 127 | 6414 | 3 | 753 | 23 | 8776 | 12 | 901 |

* THESE FIGURES INCLUDE 10 STROKE ACUTE CARE UNITS, TREATED AS A PART OF REGULAR RESEARCH GRANTS

CONTRACT NARRATIVE
Stroke and Trauma Program, NINCDS
July 1, 1977--June 30, 1976

MAYO FOUNDATION (PH 43-66-933)

Title: Bibliographic Service on Cerebrovascular Disease

Contractor's Project Director: Robert G. Siekert, M.D.

Current Annual Level of Support: \$25,000.00

Objective: To provide an abstracting service relative to various aspects of cerebrovascular disease. Contents of 150 journals are scanned. The abstracts are published in Stroke, a Journal of Cerebrovascular Disease.

Significance to the NINCDS Program and Biomedical Research: The abstracts on cerebrovascular disease continue to be a valuable service to the health profession.

Proposed Course: This contract will be extended to continue this valuable service.

CONTRACT NARRATIVE
Stroke and Trauma Program, NINCDS
July 1, 1975--June 30, 1976

INDIANA UNIVERSITY FOUNDATION (N01-NS-2-2324)

Title: Study of Hospital Frequency and Character of Transient Ischemic Attacks

Project Director: Mark L. Dyken, M.D.

Current Annual Level of Support: \$86,295.00

Objectives: The main purpose of the study was to learn what the current medical practices were and to accomplish the following objectives:
1. identify all patients with complaints or diagnosis of TIA's, 2. establish the reliability of the diagnosis, 3. determine the usual diagnostic procedures, 4. determine the types, effects, and complications of therapy, 5. determine the frequency of changes in diagnosis, new vascular events and intercurrent disease during follow-up, and 6. establish a known base which could be used for planning more definitive studies.

Major Findings: Patients with TIA-like symptomology at six different major medical institutions were identified and 954 patients were followed for a mean of 14.3 months. The reliability of the diagnosis and the treatment procedures were established. The patients were followed to determine types, effects and complications of therapy; the frequency of changes of diagnosis, new vascular events and intercurrent diseases.

Significance to NINCDS Program Area Research: Transient ischemic attacks (TIA's) are important since they frequently warn of an impending stroke and provide an opportunity for medical and/or surgical intervention in the hope of preventing a completed stroke. Failure to make a correct diagnosis in patients with TIA's can lead to incorrect treatment. The purpose of this study is to help lower the mortality and morbidity and expense associated with stroke. In addition to medical treatment, both extracranial and intracranial surgical intervention procedures are now technically available. The efficacy of any of the intervention methods depends on the reliability of the diagnosis. This study will establish criteria for the diagnosis of TIA's.

Proposed Course: The assembling and analysis of the data collected in this study will continue through July 31, 1977.

CONTRACT NARRATIVE
Stroke and Trauma Program, NINCDS
July 1, 1975--June 30, 1976

HOWARD UNIVERSITY COLLEGE OF MEDICINE (N01-NS-4-2329)

Title: A Study of Prognosis after Stroke

Project Director: Don H. Wood, M.D.

Current Annual Level of Support: \$139,006.00

Objectives: 1. To provide continuous neurological observations over a six-month period after stroke, beginning with admissions to acute treatment facility and concluding with functional assessment of quality of recovery.
2. To test prospectively in a longitudinal study the accuracy and reliability of clinical signs and symptoms reputed to have prognostic value from retrospective surveys.

Major Findings: As of February 1976, 204 patients have been evaluated of whom 114 have met entrance criteria to the longitudinal study protocol.

It has been observed that patients and families have been highly motivated and extremely willing to return for follow-up care.

The programming support for this project has, to a large extent, been dependent upon finalizing the format of our source documents (the Inpatient Hospitalization Data and the Daily and Monthly Evaluation Flow Sheet forms). With final revisions submitted for these source documents, the following programming tasks have been completed:

1. Development of program specifications for the edit/update program to create a master file (tape) consisting of Inpatient Hospitalization Data.
2. Development of program specifications for the edit/update program to create a master file (tape) consisting of Daily and Monthly Evaluation Data.
3. Coding and compilation of the computer programs referenced in items (1) and (2) above.
4. Preliminary testing of the edit/update program to create the master file of Inpatient Hospitalization Data using dummy test data.

In addition to the completed tasks mentioned above, all data for patients who have completed the first 14 days of evaluation are in the process of being entered on punched cards.

Significance to NINCDS Program and Biomedical Research: This study is a necessary step toward achievement of the goals and objectives of the Head Injury and Stroke Section's program, aimed at improvement in the quality of

life of survivors. From retrospective data analyses, coma or the state of consciousness at the outset of strokes is the most reliable prognostic indicator of both survival and the quality of recovery. This study correlates closely with the concurrent study "Prediction of Outcome of Patients in Coma" in that it takes into account the multivariants affecting outcome, while the Cornell University project attempts to precisely define and quantitate degrees of coma as well as its duration.

Proposed Course: The project will be completed within the calendar year 1976.

CONTRACT NARRATIVE
Stroke and Trauma Program, NINCDS
July 1, 1975--June 30, 1976

MAYO MEDICAL SCHOOL (N01-NS-4-2333)

Title: Study of Transverse Axial Tomography in Cerebrovascular Disease

Project Director: Otis W. Houser, M.D.

Current Annual Level of Support: \$123,080.00

Objectives: The primary aim is to compare the efficacy of computerized tomography and isotope brain scans for the detection of lesions in 250 patients with clinical stroke. A second goal is to study computer methods for analyzing the CT scans of patients with stroke.

Major Findings: One hundred twenty-eight supratentorial cerebral infarctions were clinically identified and radiologically studied in 171 patients with suspected focal cerebrovascular disease. All patients were studied by computerized tomography (CT) soon after the initial clinical evaluation, and follow-up studies were performed within 7 to 10 days. Seventy-one percent (71%) had CT scans, 74% had ^{99m}Tc scans, and 63% had both scans. A focal CT abnormality was identified in two-thirds of the infarctions (of which 90% were less than one month duration). The pattern of infarct evolution was studied in those patients having a second CT scan.

Approximately 5% of patients had an initially negative scan which became positive on the follow-up examination; all initial scans in this group were performed within 48 hours of the initial event. A similar number of patients had an initially positive scan which reverted to negative. Some degree of mass effect was identified in 40% of positive CT scans obtained within two weeks of the initial event. In addition to evolving changes, failure to visualize a focal CT abnormality was correlated with the presence of minimal neurologic deficit, the clinical suspicion of a small, deep, or parasagittal lesion, and the radiographic identification of cerebral atrophy or movement artifact on the CT scan. Fifty-three percent (53%) of the ^{99m}Tc scans performed in 94 patients also showed a focal abnormality.

Although the CT scan had only a slightly more favorable detection rate for cerebral infarction, it was of major value and superior to the ^{99m}Tc scans in differentiating between infarct, intracerebral hemorrhage, neoplasm, and other intracranial pathology.

Significance to NINCDS Program and Biomedical Research: Successful completion of this study will provide the NINCDS with a true test in terms of real pathology of the spatial resolution, temporal latency, and thereby the limits of applicability of this new technology to clinical stroke problems. While a body of literature has developed very quickly on the sensitivity of CAT scans to intracerebral hemorrhages, little has been spontaneously reported on the utility of the equipment in detecting and localizing acute ischemic processes.

Data and conclusions from this study will provide guidelines for the appropriate application of CAT techniques in existing and future comprehensive cerebrovascular disease research programs.

Proposed Course: This project will be completed by the end of FY-77.

CONTRACT NARRATIVE
Stroke and Trauma Program, NINCDS
July 1, 1975--June 30, 1976

DUKE UNIVERSITY MEDICAL CENTER (N01-NS-4-2337)

Title: Detection of TIA in an Elderly Community

Project Director: Albert Heyman, M.D.

Current Annual Level of Support: \$84,925.00

Objectives: 1. To develop, field test and validate a self-administered questionnaire for the identification of previously undiagnosed transient ischemic attacks (TIA) in the community. 2. To assess the incidence and prevalence of TIA in the high stroke risk populations of aging Americans in a demographically selected cross section of the country.

Major Findings: The questionnaire designed to detect TIA in elderly population groups has now been distributed to some 7,245 persons over 60 years of age living in some 30 retirement facilities across the country. Medical centers in six cities have already participated in this project and two remaining areas are to be studied in the next few months. The questionnaire was completed by 79.2% of the eligible persons in the six cities. It appeared to screen out some 94% of the elderly population, leaving only 6% of the respondents with evidence of transient focal neurologic deficits requiring further evaluation by the clinician.

The frequency of probable TIA as determined by the questionnaire was greater among persons 69-79 years of age than those 80 years of age or more, and more frequent among hypertensives than among those without this condition. Similarly, TIA's were reported more often by those with heart disease and stroke than by those without either of these two conditions. There was no difference in the proportion of TIA among those with and without diabetes.

The results of this project support the objective stated in the original contract proposal, i.e., to design a self-administered questionnaire which will effectively identify persons with TIA in large population surveys.

Significance to NINCDS Program and Biomedical Research: This study complements and extends the effort initiated in the collaborative hospital-based study of TIA aimed at early detection of the stroke prone. The hospital study questions the accuracy of the initial diagnosis, grades the quality and reliability of individual historical symptoms and symptom complexes in terms of short-term prognosis and final diagnosis in a preselected population presenting at or referred to university hospitals. The questionnaire developed surveys in a high risk population, unselected except by age, the most prevalent symptoms suggesting TIA and those commonly confused with TIA. Special inquiry is being made into the incidence, prevalence and significance of a reputedly common and troublesome symptom among aging persons, namely, non-vertiginous dizziness.

Proposed Course: It is planned that this phase of the study on Detection of TIA in the Elderly Community will be completed in the forthcoming year. The last of the field studies is scheduled for mid-summer 1976, following which all of the data needed for computer analysis will be on hand. Full attention will than be given to processing and analysis of this data using coding forms and computer programs which are now being designed and tested.

In addition, a small study will be carried out to investigate possible differences in the health of questionnaire respondents and non-respondents. This project will consist of a review of the medical records of the Mayo Clinic and hospitals in Olmstead County, Minnesota, for each person in the Rochester population sample entered in this study last summer.

Finally, it is proposed to carry out a new but closely related project which represents a logical development of the present study. The questionnaire which is now being validated to determine the prevalence of TIA will be applied to a free-living population. The Department of Neurology at the University of California, Irvine, will collaborate in this project which will consist of a survey of TIA in elderly persons living in the Leisure World retirement community of Laguna Hills, California. This study is regarded as an extension of the ongoing research.

CONTRACT NARRATIVE
Stroke and Trauma Program, NINCDS
July 1, 1975--June 30, 1976

ALBERT EINSTEIN COLLEGE OF MEDICINE OF YESHIVA UNIVERSITY (N01-NS-5-2305)

Title: Oxidative Metabolism in Cerebral Ischemia

Project Director: Jack M. Fein, M.D.

Current Annual Level of Support: \$39,915.00

Objectives: 1. To test in vivo the quantitative alterations in O₂ metabolism induced by specific anesthetic agents and in neurosurgical procedures. 2. To test the potential for prolongation of operative "safe time" by barbiturate drugs. 3. To test the potential efficacy of cerebral embolectomy (endarterectomy) in management of acute strokes. 4. To pretest the potential efficacy of revascularization of the reversibly ischemic field distal to the clips placed in on the MCA of the dog.

Major Findings: The local cortical and subcortical oxygen extraction rate and corresponding electrocorticogram (ECoG) were studied in primate brain before and after middle cerebral artery (MCA) occlusion. Oxygen sensitive platinum electrodes were implanted stereotactically and after spontaneous oxygen availability waves were recorded transient carotid occlusion produced a decay whose slope was related to the extraction rate. Under nitrous oxide/oxygen anesthesia the local oxygen extraction slope (LOES) for the frontal cortex was $1.95 \pm .71$, parietal cortex $1.99 \pm .42$, opercular cortex $1.92 \pm .55$ and centrum semiovale $.96 \pm .15$. Simultaneous ECoG disclosed a mixture of 2 - 6 c.p.s. activity, 80 - 140 μ v amplitude. The immediate response to MCA occlusion in 7 monkeys was a reduction of LOES in opercular cortex to $.15 \pm .02$ and in subcortical white matter to $.09 \pm .01$. Low voltage slow activity was noted in a corresponding distribution. Within one hour of occlusion 9 of 23 peripheral electrodes in frontal and parietal cortex showed a significant increase in LOES ($p < .001$) which was sustained until sacrifice one week after occlusion. Peripheral cortical ECoG showed the development of high voltage slow activity with prominent spike and sharp wave activity which correlated with areas of increased LOES.

Cortical areas surrounding an ischemic zone may have an increased neuronal activity. The "luxury perfusion" seen and described by others surrounding a cerebral infarction may be a reflexion of the increased metabolic requirements of these irritable foci.

Significance to the NINCDS Program and Biomedical Research: These studies will help provide a potential scientific foundation for the existing superficial temporal middle cerebral artery bypass procedures and for the role of barbiturate protection in segmental arterial occlusion (focal cerebral ischemia).

Recent experience with microvascular surgical techniques in cerebral ischemia

problems has failed to give a clear cut answer to the efficacy of this mode of therapy. More detailed information regarding local tissue blood flow and oxygen extraction may be helpful when determined during the ischemic process following anastomotic corrections and under the influence of barbiturate anesthesia and metabolic inhibitors.

With new information and increased insight into these fundamental aspects of ischemic cerebral tissue it is possible that microvascular surgery in various thrombotic and embolic conditions can be made more effective.

Proposed Course: The contract will continue through December 31, 1976.

CONTRACT NARRATIVE
Stroke and Trauma Program, NINCDS
July 1, 1975--June 30, 1976

UNIVERSITY OF MARYLAND SCHOOL OF MEDICINE (N01-NS-5-2310)

Title: Capillary Angiogenesis and Neuronal Viability in Acute Cerebral Ischemia

Project Director: Julio H. Garcia, M.D.

Current Annual Level of Support: \$33,000.00

Objectives: 1. Measure levels of endogenous and radioactively labeled tyrosine, tyramine, dopamine and norepinephrine at sequential intervals (0-72 hrs.) after occlusion of a middle cerebral artery in primates (*Maccaca mulatta*). 2. Conduct parallel morphological (ultrastructural) studies of the same experimental model in order to correlate the dynamic and regional properties of the lesions with those characterizing the abnormal anatomy. 3. Chronic studies (1-6 weeks) of similar lesions in a second group of primates.

Major Findings: 1. Measurement of known quantities of tyrosine in standard solutions has been accomplished by the application of spectrofluorometric assays of nitrosonaphthol derivatives of tyrosine. 2. The successful separation of dopamine and tyrosine previously mixed in a common solution by the use of ion exchange chromatography. 3. Measurement of dopamine and tyrosine in mammalian brains.

Significance to NINCDS Program and Biomedical Research: These experiments demonstrate changes in capillary permeability. These changes have significance for experiments which are attempting to reduce the infarction associated cerebral edema.

Proposed Course: The contractor plans to complete the series of combined experiments including occlusion, injection of C-14 tyrosine and determination of its derivatives in the ischemic and nonischemic tissues. It will be done at increasingly longer ischemic intervals and there will also be parallel studies to characterize the nature of the structural abnormalities developing in brains of animals treated in a similar fashion.

CONTRACT NARRATIVE
Stroke and Trauma Program, NINCDS
July 1, 1975--June 30, 1976

UNIVERSITY OF WASHINGTON (N01-NS-2-2326)

Title: Human Responses to Head Injury

Project Director: Ralph M. Reitan, Ph.D.

Current Annual Level of Support: \$30,724.00

Objectives: To establish the following: 1. To what degree a patient recovers various aspects of psychologic function following head injury. 2. If there is differential recovery with regard to various parameters measured. 3. If emotional improvement parallels intellectual improvement. 4. To what extent psychologic dysfunction may be related to neurologic and EEG findings. 5. Which aspects of neurologic, EEG or psychologic data have predictive significance regarding return of function.

Major Findings: 1. At the end of the third year of the study there were 34 patients who had completed their 18-month follow-up examinations. 2. Eleven (11) of these 34 showed no evidence of significant brain dysfunction. 3. Improvement in neuropsychological function is most marked in the first 12-month period. Improvement after this occurs but to a lesser extent. 4. Twenty-five percent (25%) of the subjects showed psychologic deterioration between the twelfth and eighteenth month, even though they had improved during the first year. There has been no follow-up of these few patients past eighteen months. 5. A statistical study comparing three groups, one with head injury and epilepsy, one with head injury, epilepsy and other neurologic dysfunction and one of normal control subjects. The results suggested that head injury complicated by epilepsy, even without neurologic deficit, was associated with a broad range of adaptive disabilities.

Significance to NINCDS Program and Biomedical Research: Because of the small number of patients and the lack of normal controls, it is doubtful that many conclusions can be drawn from this study. However, Dr. Reitan and Dr. Dickman will submit a final report.

Proposed Course: This contract terminated on May 31, 1976.

CONTRACT NARRATIVE
Stroke and Trauma Program, NINCDS
July 1, 1975--June 30, 1976

UNIVERSITY OF MINNESOTA (N01-NS-4-2306)

Title: Comparison of Isotope-Bolus Technique with Contrast and Isotope Angiography

Project Director: Shelley N. Chou, M.D.

Current Level of Annual Support: 0

Objective: To test a simple, portable, intravenous isotope-bolus technique for demonstrating the absence of cerebral perfusion by comparison with both isotope and contrast cerebral angiography.

Major Findings: Accession rate has remained low. Dr. Chou attributes this to the reduced speed limits in Minnesota. He states that at present there are approximately 20 patients in the study. One of these was a case of brain death secondary to barbiturate intoxication. The bolus study and the angiography in that case revealed flow. However, in the other cases the bolus study seemed to be a good indicator for lack of cerebral perfusion.

Significance to NINCDS Program and Biomedical Research: Cerebral angiography has remained the most reliable method for demonstrating cerebral circulation. Four-vessel cerebral angiography has been used in Europe as one of the criteria for establishing the diagnosis of brain death. However, this procedure involves both the risk of transporting the moribund patient and his support equipment to the x-ray department and the risk of the technique itself. Therefore, if the isotope-bolus technique can be shown to be as reliable as angiography in determining the absence of cerebral perfusion, a simple, portable and relatively safe procedure will be available for assessing brain death.

Proposed Course:

Dr. Chou has requested an extension without funds until June 24, 1977.

CONTRACT NARRATIVE
Stroke and Trauma Program, NINCDS
July 1, 1975--June 30, 1976

NEW YORK UNIVERSITY (N01-NS-4-2307)

Title: Intravenous Isotope Scintillation Angiography of the Brain in Man

Project Director: Philip Braunstein, M.D.

Current Annual Level of Support: 0

Objective: Validation of the radioisotope method of assessing cerebral circulation.

Major Findings: Twenty (20) patients have been studied with angiography and bolus techniques. All showed no significant intracranial blood flow by angiography. Nineteen (19) had flat or intermediate bolus studies. One (1) had a small bolus effect. This patient also had a bizarre angiogram with dye retained in the area of the superior sagittal sinus. The investigators offer the following conclusions: 1. Apart from one case which is not well understood there has been no case in which the bolus study was considered inaccurate. 2. "Intermediate" and "no bolus" have the same meaning, i.e., no significant intracranial blood flow. 3. The bedside intravenous bolus injection is a good way to establish brain death. A final report and paper are in progress.

Significance to the NINCDS Program and Biomedical Research: Cerebral angiography has remained the most reliable method for demonstrating cerebral circulation. Four-vessel cerebral angiography has been used in Europe as one of the criteria for establishing the diagnosis of cerebral death. However, this procedure involves both the risk of transporting the moribund patient and his support equipment to the x-ray department and the risk of the technique itself.

The present study suggests that the isotope-bolus technique may be as reliable as angiography in determining the absence of cerebral perfusion and thus may provide a simple, portable and relatively safe procedure for assessing brain death.

Proposed Course: This contract terminated on December 31, 1975. Final report is still awaited.

CONTRACT NARRATIVE
Stroke and Trauma Program, NINCDS
July 1, 1975--June 30, 1976

CORNELL UNIVERSITY MEDICAL COLLEGE (N01-NS-4-2328)

Title: Prediction of Outcome of Patients with Coma

Project Director: Fred Plum, M.D.

Current Annual Level of Support: \$65,026.00

Objectives: 1. To test the accuracy and validity of a semiquantitative system for grading coma. 2. To assess the predictive power of each indicant with regard to short term prognosis (3 months).

Major Findings: Currently there are 1,130 cases in the study, 284 medical and 846 surgical. In a report received in February 1975 titled "The outcome of medical coma: prediction by bedside assessment of physical signs" the cases of 115 patients in medical coma were reviewed. It was noted that on admission the sign giving the single best accuracy of prediction was the oculovestibular reflex. Papers are currently in progress on 700 cases of severe head injuries, and one paper, "Predicting outcome in individual patients after severe head injury" will appear in Lancet.

Significance to the NINCDS Program and Biomedical Research: The large number of cases in this study will permit further testing of a semiquantitative system for grading the severity of coma. The general acceptance of such a scale would facilitate neurologic communication and aid greatly in discussions of stroke and head injury.

This study will give some idea not only of the percentage, but also the quality, of survival of patients in coma. Finally, if a particular sign or signs elicited on initial examination permit the neurologist to predict with some certainty the nature of the outcome of the individual case, this would have broad implications with regard to the type and duration of treatment.

Proposed Course: This project is funded until June 29, 1977.

CONTRACT NARRATIVE
Stroke and Trauma Program, NINCDS
July 1, 1975--June 30, 1976

UNIVERSITY OF PENNSYLVANIA (N01-NS-5-2316)

Title: Computerized Axial Tomography in Acute Head Injury

Project Director: David E. Kuhl, M.D.

Current Annual Level of Support: \$203,016.00

Objectives: 1. To determine the ability of computerized axial tomography to detect and distinguish lesions in acute head injury. 2. To test the ability of CAT to predict delayed cerebral manifestations of closed head injury. 3. To determine the optimal time for CAT in management of acute severe head injuries.

Major Findings: This study has been in progress less than a year. Although the initial accession rate was slow, this has improved. At present 95 patients have been admitted to the study. Both x-ray and radionuclide computerized tomography have been used, x-ray CT on 144 occasions and radionuclide on 67. Fifteen (15) cerebral angiograms have been done. Emphasis is being placed on two areas: 1. the pediatric concussion syndrome, 2. longitudinal studies in intracerebral hemorrhage and subdural collections. Dr. Kuhl is enthusiastic with regard to the delineation of intracranial lesions by CAT. It is, however, too soon in the study to discuss the significance of their findings.

Significance to NINCDS Program and Biomedical Research: CAT is one of the most sensitive techniques available for localization and identification of intracranial lesions. It is, however, the latest tool in the armamentarium of the neurologist and neurosurgeon and as such needs evaluation and comparison with more established techniques such as angiography. For decades cerebral angiography has offered the most reliable information in ruling out intracranial hematomas. If comparable information can be obtained from CAT, a definite service will have been rendered to the head-injured patient. Since CAT does not involve arterial puncture and in many cases does not involve the use of the contrast agent, it carries significantly less risk than angiography. Initial evaluation of the patient would therefore be safer and the physician would more readily order serial examinations to follow the patient's course.

Proposed Course: This contract is funded until June 29, 1977.

CONTRACT NARRATIVE
Stroke and Trauma Program, NINCDS
July 1, 1975--June 30, 1976

UNIVERSITY OF NEW MEXICO (N01-NS-5-2332)

Title: Quantitative Intracranial Pressure Measurement in Man

Project Director: A. Earl Walker, M.D.

Current Annual Level of Support: \$121,487.00

Objective: To evaluate clinically in human subjects a system for monitoring intracranial pressure.

Major Findings: Production of stable transducers has been a major problem. Transducers which have passed in vitro stability tests have shown good correlation in vivo between the pressure they indicate and pressure recorded in spinal tap.

Significance to the NINCDS Program and Biomedical Research: A system capable of detecting increases in intracranial pressure before clinical signs manifest themselves would permit better and faster treatment of patients with intracranial lesions.

Proposed Course: The system will be evaluated in many more patients if stable transducers can be obtained.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE
PROJECT NUMBER (Do NOT use this space)

U.S. DEPARTMENT OF
HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE
NOTICE OF
INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 NS 02118-03 STP

(Prev.No.:Z01 NS 02118-02 ANR)

PERIOD COVERED

July 1, 1975 through June 30, 1976

TITLE OF PROJECT (80 characters or less)

Stroke Models in Primates--Intracerebral Hematoma

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER
PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

P.I: John P. Laurent, M.D., University of Pennsylvania, Philadelphia, Pa.

O.I: G. F. Molinari, M.D., Chairman, Dept.of Neurol.,Geo.Wash.U.,Wash.,D.C.
John C. Oakley, M.D., Univ. of Washington, Seattle, Wash.

All investigators were formerly with Section on Head Injury and Stroke,ANR,NINCDS

COOPERATING UNITS (if any)

None

LAB/BRANCH

SECTION

INSTITUTE AND LOCATION

NINCDS, NIH, Bethesda, Maryland

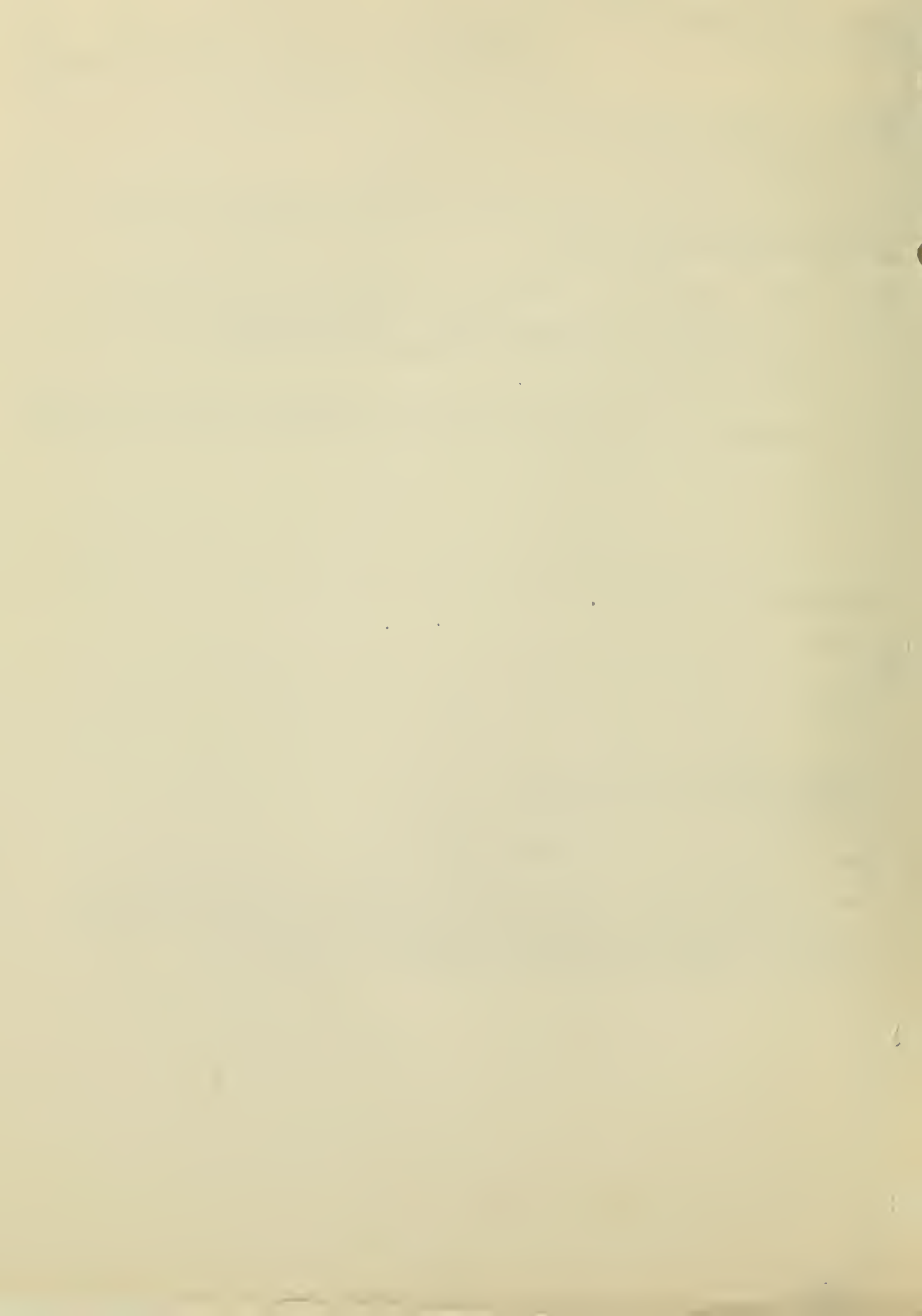
TOTAL MANYEARS:

PROFESSIONAL:

OTHER:

SUMMARY OF WORK (200 words or less - underline keywords)

The Objectives, Methods Employed and Major Findings were described in our previous report--July 1, 1974 through June 30, 1975. This project was completed in August 1975 and the findings will be published in the Journal of Neuropathology and Experimental Neurology in November 1976.



| | | |
|--|---|---|
| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 NS 02120-03 STP (Prev.No.:Z01 NS 02120-02 ANR) |
| PERIOD COVERED July 1, 1975 through June 30, 1976 | | |
| TITLE OF PROJECT (80 characters or less) Evaluation of the Role of Barbiturate in Treating Acute Cerebral Infarction | | |
| NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT P.I: G. F. Molinari, M.D., Chairman, Dept. of Neurol. Geo.Wash.U.,Washington,D.C. O.I: John P. Laurent, M.D., Univ. of Pennsylvania, Philadelphia, Pennsylvania John C. Oakley, M.D., Univ. of Washington, Seattle, Washington All investigators were formerly with Section on Head Injury and Stroke, ANR, NINCDS | | |
| COOPERATING UNITS (if any) None | | |
| LAB/BRANCH | | |
| SECTION | | |
| INSTITUTE AND LOCATION NIH, NINCDS, Bethesda, Maryland | | |
| TOTAL MANYEARS: | PROFESSIONAL: | OTHER: |
| SUMMARY OF WORK (200 words or less - underline keywords) The Objectives, Rationale, and Major Findings were described in our previous report--July 1, 1974 through June 30, 1975. This project has been completed and was presented at the Joint Meeting on Stroke and Cerebral Circulation, Dallas, Texas, February 27-28, 1976. Molinari GF, Oakley JC, Laurent JP: The pathophysiology of barbiturate protection in focal ischemia. Stroke 7:3-4 Jan.-Feb. 1976 (Abstract) | | |

ANNUAL REPORT
July 1, 1975 through June 30, 1976
Communicative Disorders Program
National Institute of Neurological and
Communicative Disorders and Stroke

Introduction

The Communicative Disorders Program of the National Institute of Neurological and Communicative Disorders and Stroke is concerned with research on improving the diagnosis, treatment, and prevention of diseases and disorders which affect the ear, nose and throat and cause problems relating to hearing, language, and speech. The disorders of human communication are some of the most frequent disabilities in our society, for approximately 10 percent of the population, or over twenty million individuals, are so affected. Of these disorders, hearing loss is the most prevalent. The most recent census of the deaf indicates between thirteen and fourteen million with sufficient hearing loss to significantly affect their ability to function effectively in everyday activities. Since such a defect is not visible and usually is not a cause of death, the problem is often downgraded. However, one cannot overlook the economic factors of: cost for special education and rehabilitation; and loss of potential earning power for self support. Even more important for the individual and the family involved is the change in the quality of life caused by such disabilities.

Formation of the Communicative Disorders Program

The formation of the Communicative Disorders Program was a part of the attempt of the NINCDS to focus greater emphasis on the problems described above. An initial step in giving greater visibility to communicative disorders was the change in the name of the Institute in March 1975 from National Institute of Neurological Disorders and Stroke to National Institute of Neurological and Communicative Disorders and Stroke. Following the name change, a reorganization within the Institute was undertaken to establish four program areas, one of which was the Communicative Disorders Program area. The announcement of the reorganization appeared in the Federal Register on May 27, 1975, but it was actually nearly a month later (i.e., the beginning of the current year on July 1, 1975) before the reorganization became functionally a reality. Therefore, the true beginning of the present Communicative Disorders Program quite closely coincides with the beginning of the year covered by this Annual Report.

Prior to the reorganization, communicative disorders was represented in the NINCDS as a Section under the Collaborative and Field Research Program. The Section was established in April 1972 to develop and carry out research directed toward the improvement in diagnosis and treatment of disorders of hearing, speech and language. In the ensuing three years, the Section was enlarged to include a Head, Dr. Lois Elliott, and two other staff members--Dr. Christy Ludlow and Dr. Edward Cudahy. Dr. Elliott's primary area of interest was auditory and psychoacoustics, while Dr. Ludlow was primarily involved in the area of speech and language. Dr. Cudahy was appointed as a

Staff Fellow to work with Dr. Elliott on research problems in psychoacoustics. Secretarial support for the Section members was provided by Mrs. Mary Harmon. At the time of the reorganization, it was this staff from the former Communicative Disorders Section which was transferred with the Communicative Disorders Contract Program they had developed to provide one of the major components of the new Communicative Disorders Program.

A second component of the new Communicative Disorders Program came from the former Extramural Programs area. All of the individual research grants, the program projects, and the clinical centers related to communicative disorders were transferred to the new Communicative Disorders Program. Dr. Irving Woods, the Health Scientist Administrator who had been most intimately involved with these projects in the Extramural Programs, was also transferred with his secretarial staff, Ms. Frances Chandler, to the Communicative Disorders Program.

The third component of the new Communicative Disorders Program came as a result of the appointment of a new Director to head the Program, Dr. Wesley H. Bradley. Dr. Bradley, an otolaryngologist, came to the NINCDS from Syracuse where he had practiced clinical otology and served as clinical professor of otolaryngology at the Upstate Medical Center of the State University of New York. Mrs. Beverly Surles, formerly secretary to the Chief, Office of Scientific and Health Reports, NINCDS, assumed the position of secretary to Dr. Bradley in August 1975. Thus, with the amalgamation of these three components, the present Communicative Disorders Program was born almost concurrently with the beginning of the current fiscal year.

The new Communicative Disorders Program provides a focus within the NINCDS for all activities related to communicative disorders, whether they be directed research such as contracts or investigator-initiated research such as individual research grants, program projects, or clinical centers. The logical further development to be anticipated is that this programmatic approach will allow the staff to gradually become familiar with all mechanisms for supporting research and that greater flexibility in utilizing the appropriate mechanisms will thus result. In addition, this consolidation of all communicative disorders activities within the program area provides a focus for information delivery, cooperation with other Institutes or other Governmental agencies, and cooperation with professional organizations related to communicative disorders which are outside the Government. Both time and adequate staff will be required to eventually fully achieve these ends.

Changes in Staff

Only shortly after the Program staff was consolidated, a major change occurred. Dr. Elliott was offered and accepted an important position in her professional area at Northwestern University. Our pride in her selection and our happiness for her personally was counter-balanced by the loss of her efforts and energies within the program area. Dr. Elliott left the Program in early December 1975, and as of this date a replacement has not been obtained. Active recruitment is under way and it is hoped that an individual will be obtained before the remainder of this year is concluded. The shortage of staff in this key position has been most crucial, and has limited all aspects of the program, but particularly in the area of directed research.

Changes in Geographic Location

In an attempt to make the Institute reorganization by program areas more functional, the location of all the program areas within one building, the Federal Building, was determined to be highly desirable. For the Communicative Disorders Program, this required the transfer of the three segments from: Building 31, Building 36, and the Westwood Building. The long anticipated move was consummated in February 1976 with all elements of the program, except for the research areas, consolidated on the first floor of the Federal Building. Dr. Ludlow and Dr. Cudahy continue to use space in Building 36 for their individual research projects. Although the time since the move is still brief, the consolidation already appears to have unified the program. The opportunity for interaction within the staff of the Communicative Disorders is greatly enhanced. It is hoped that similar increased interaction among the program areas will also result as a consequence of the move.

Research Grant and Contract Activities

A more detailed picture of these areas is included further on in this report. A more general picture of the research grant program in the communicative disorders area can be obtained from material prepared for the March 1976 Meeting of the National Advisory Council of the NINCDS. At that time, all active grants in the program area were compiled and categorized into one of three major categories as in Figure 1 below.

Figure 1

| | <u>GRANTS</u> | <u>DOLLAR VALUE</u> |
|-------------------------|---------------|---------------------|
| I. HEARING AND BALANCE | 168 | \$14,066,270 |
| II. SPEECH AND LANGUAGE | 41 | 3,201,712 |
| III. SPECIAL SENSES | 89 | 4,471,436 |
| TOTAL | 298 | \$21,739,418 |

The further breakdown of these major categories is shown in Figures 2, 3, and 4.

Figure 2

| | <u>GRANTS</u> | <u>DOLLAR VALUE</u> |
|------------------------|---------------|---------------------|
| I. HEARING AND BALANCE | | |
| HEARING | 140 | \$12,244,666 |
| BALANCE | 28 | 1,821,604 |
| TOTAL | 168 | \$14,066,270 |

Figure 3

| | <u>GRANTS</u> | <u>DOLLAR VALUE</u> |
|-------------------------|---------------|---------------------|
| II. SPEECH AND LANGUAGE | | |
| SPEECH | 30 | \$2,349,855 |
| LANGUAGE | 11 | 851,857 |
| TOTAL | 41 | \$3,201,712 |

Figure 4

| | <u>GRANTS</u> | <u>DOLLAR VALUE</u> |
|---------------------|---------------|---------------------|
| III. SPECIAL SENSES | | |
| TASTE | 15 | \$ 722,881 |
| TOUCH | 18 | 833,336 |
| SMELL | 18 | 1,002,627 |
| PAIN | <u>38</u> | <u>1,912,592</u> |
| TOTAL | 89 | \$4,471,436 |

The main points to be derived from this material are: (1) Otolaryngology grants per se do not appear in this breakdown, but only as portions of the general groupings of hearing, speech and language; (2) the grants related to special senses are a major part of the research grant program as presently coded (approximately 20 percent); and (3) the total dollar volume of the grant program appears larger than the outside communicative disorders community has believed--again because of the size of the investment and grants related to special senses.

In the contract area, both the total number of communicative disorders contracts and the dollar investment will appear significantly less compared to the preceding year. Part of this is due to the more appropriate coding of some contracts previously coded as communicative disorders, but now changed to Fundamental Neurosciences, in the Neural Prosthesis Program. A second factor has been that because of the shortage of staff mentioned earlier and changes in program relevance, fewer new starts in contracts have been initiated this year.

Special Program Activities

At the beginning of the current year (June 30 - July 1, 1975) a Workshop on Tactile and Visual Aids for the Deaf was held at NIH under the sponsorship of the Communicative Disorders Program, NINCDS. Dr. Elliott served as Chairman and Program Coordinator of the Workshop which included approximately 25 invited individuals with experience in this area. Among the recommendations of the Workshop the following were especially noted: (1) the need for additional test materials for evaluating sensory aids, in particular those that combine tactile or visual presentations with lipreading; and (2) for devices already developed, a program of field trials that involves an appropriate sampling of the target population, documented training procedures, and adherence to standard evaluation procedures.

This year saw the phase out and final closing of the Information Center for Hearing, Speech, and Disorders of Human Communication at The Johns Hopkins Medical Center. The action came as the result of recommendations of a site visit team, the Science Information Program Advisory Committee (SIPAC) of NINCDS, and the NINCDS National Advisory Council. In an attempt to investigate other possibilities for providing information service to the communicative disorders community, the staff of the Communicative Disorders Program has investigated the services of the National Library of Medicine. A preliminary survey suggests that some enrichment of the National Library of Medicine data base and incorporation of better descriptors into the

National Library of Medicine system might achieve a very usable system for communicative disorders at a significantly lower cost. This possibility is now being further investigated.

Special attention should be called to the three volume set entitled The Nervous System published during this current year under the editorship of Dr. Donald Tower as a part of the 25th Anniversary Celebration of the NINCDS. Volume III is devoted completely to the topic of human communication and its disorders. It is a high quality representation of the work of many individuals who have been involved in various ways with the communicative disorders programs of the NINCDS during the past quarter of a century.

Activities of the Professional Staff

Members of the staff have participated in numerous professional activities outside the NINCDS program, some of which are noted below,

Dr. Bradley served as Program Chairman for one of the sessions of the International Workshop on Middle Ear Surgery and Fluctuant Hearing Loss in Chicago in February 1976. He will moderate a panel during the International Conference on Cholesteatoma in Iowa City in May 1976. In November 1975 as a Director of the American Board of Otolaryngology he participated in the Annual Certifying Examination. He served as a Member of the Council of both the Academy of Ophthalmology and Otolaryngology and the American Otological Society during the current year. As President of the Otosclerosis Study Group, he will have charge of the program for the Annual Meeting of the Society to be held later in the year.

Dr. Ludlow presented a paper entitled "Recovery from Aphasia: A Foundation for Treatment" in April 1976 in Omaha as part of a Symposium on the subject "Rationale for Adult Aphasia Therapy."

Dr. Cudahy presented two papers, both co-authored with Dr. Elliott, before the Acoustical Society of America. The first was entitled, "Temporal Processing in Noise by Persons With Noise-Induced and Age-Related Hearing Loss" and was presented at the November 1975 Meeting. The second was entitled, "Temporal Masking Patterns for Hearing-Impaired and Normal Listeners" and was presented at the April 1976 Meeting.

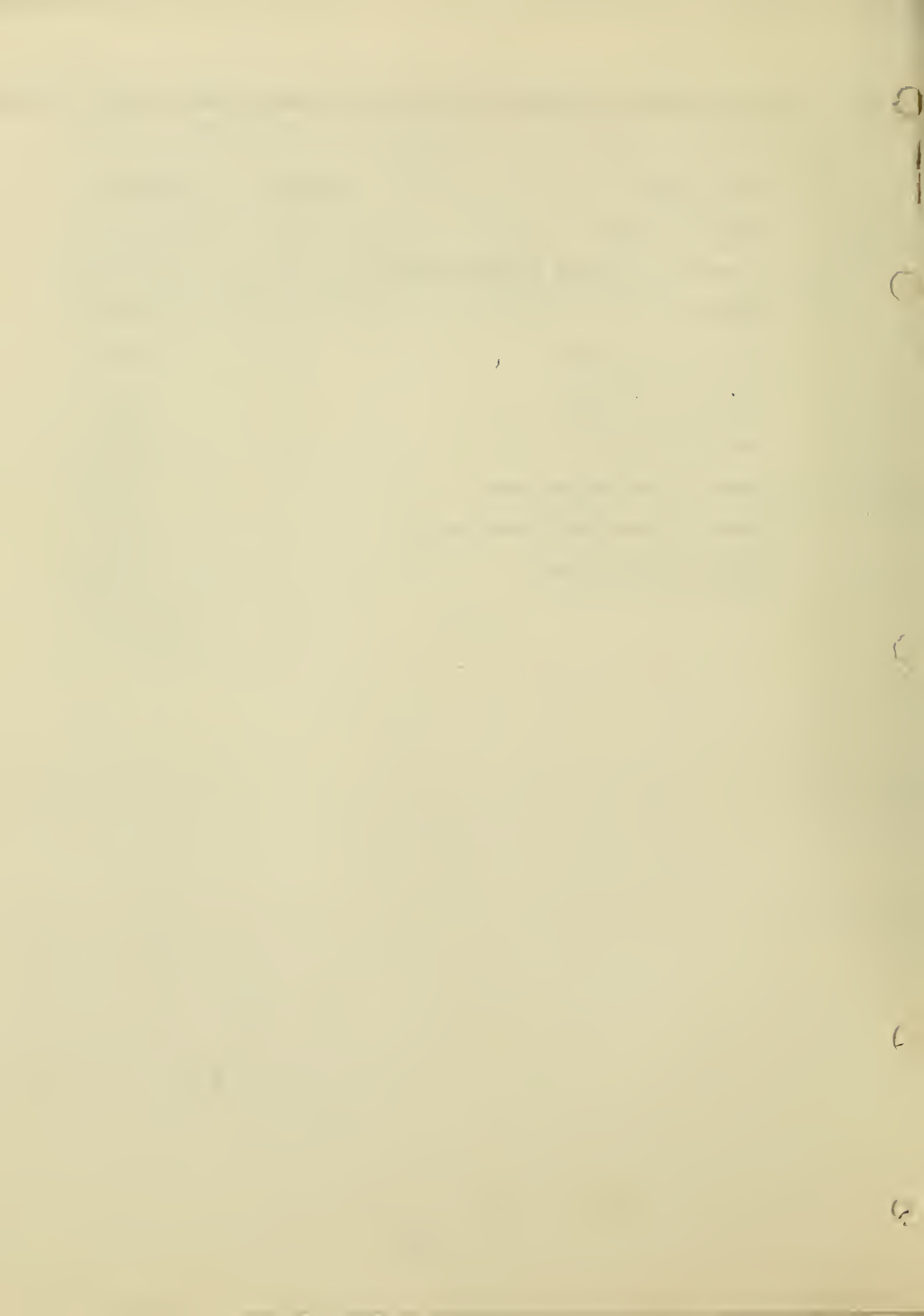
Future Projections

This year has been a period of reorganization, regrouping, and taking stock for the Communicative Disorders Program. The immediate major problem is personnel replacement to provide the coverage within the staff which has been lacking during a major portion of this year. When staff can be brought to full strength, then a more complete analysis of the program can be achieved which should eventually provide for a closer awareness of the total investigator-initiated portion of the program. Such an awareness should indicate leads as to where directed programs may be appropriate, and provide the staff personnel to develop and monitor such programs.

In a longer-term projection, the possibility of involvement of otolaryngology and the communicative disorders in the programs at the Clinical Center should be raised. Especially with the ambulatory care center close to becoming a reality, the opportunity for such expansion in the program becomes a real probability. Again, to provide such a service will require staff whose major responsibility will be for such a clinical program. The planning for such a program and the support to bring it about should be anticipated even at this seemingly early date. The establishment of the new Uniformed Services Medical School across the street on the grounds of the Naval Medical Center could perhaps provide possibilities for cooperation which would assist in the development of such a clinical program.

Number of Awards and Dollars Expended by the Communicative Disorders Program

| <u>Type of Award</u> | <u>Number</u> | <u>Amount</u> |
|---------------------------------------|---------------|---------------|
| Research Grants | 219 | \$11,154,000 |
| Program Projects and Clinical Centers | 16 | 6,027,000 |
| Contracts | 16 | 634,000 |
| Old Training Grants | 13 | 753,000 |
| New Training Grants | 5 | 293,000 |
| New Fellowships | 31 | 419,000 |
| Teacher-Investigator Awards | 3 | 67,000 |
| Research Career Development Awards | 6 | 139,000 |
| Research Career Awards | 2 | 63,000 |
| | <hr/> | <hr/> |
| TOTAL | 311 | \$19,549,000 |



Grants Activity Summary
Communicative Disorders Program

Introduction

The NINCDS is continuing to support research in the biomedical sciences and clinical investigations leading toward the goal of better understanding of the mechanisms of normal communication and those diseases which impair it. This year has been highlighted by many valuable contributions.

One of the most valuable has been the three volume work commemorating the 25th anniversary of the NINCDS, edited by Dr. Donald B. Tower. Particularly valuable to works in the Communicative Sciences is Volume III, Human Communications and its Disorders. This volume editor was Dr. Eldon L. Eagles. There are 51 self-contained chapters by internationally known authors and the chapters represent a small state-of-the-art report. It covers virtually every field of hearing, balance, speech and language, including anatomy, physiology, biochemistry, trauma, infections, and a small group of chapters devoted to central processing.

In reviewing these chapters it is evident that in nearly every one the research has been made possible or influenced by work supported by the NINCDS.

Hearing and Deafness

In a research effort directed at obtaining information which will help professionals to guide hearing-impaired persons in the proper choice of hearing aids and in the effective use of hearing aids in everyday life, a team is exploring for interactions between hearing impairment, acoustic features of the listening environment, binaural hearing aid efficiency and physical characteristics of hearing aids. The specific objective is to study conditions during which binaural fusion is retained by persons with sensori-neural hearing loss and the degree to which fusion is disturbed (if it is) by hearing aid use. Unaided and aided performance of normal hearing subjects is the reference against which performance of the hearing-impaired will be evaluated. Results of two experiments are given. When binaural signals are presented diotically to subjects with normal hearing, the listeners perceive a unitary image located in the center of the head, that is, the two signals fuse into a single percept. However, it is unclear as to whether this same experience occurs when a person has a sensori-neural hearing loss and is wearing a hearing aid. Thus, a main object is to determine the degree to which sensori-neural hearing loss and the use of hearing aids disrupts binaural fusion. But base line data on normals were first sought. Data demonstrated aural fusion existing under defined experimental conditions. However, as interaural time disparities are imposed between binaural speech signals, then the subject's result indicates that the image has shifted to one side of the head. Since a shift occurs for relatively small interaural time differences, it is apparent that the central auditory system is sensitive to the interaural time relationship of the binaural signals.

Another group has investigated the biologically relevant information represented in the acoustic waveform of an avian vocalization. To this end they developed procedures for recording from the cochlear nuclei in the redwinged blackbird. To date they recorded responses from 150 cells in nucleus angularis and nucleus magno-cellularis. Using simple stimuli (tones, noise and clicks), many cells respond only with an increase in discharge rate during the tone, other cells respond to a central range of frequencies which increased discharge rates; tones on either side of this excitatory band causes a reduction in rates. In other cells the situation is reversed: there is a center region of inhibition and a surrounding region of excitation. Temporal patterns vary considerably among the cells which show inhibition. They have completed audiogram measurements for nine redwing blackbirds and three cowbirds. Audiograms are similar for both species. For both, the most sensitive frequency is about 2 kHz with a threshold between 5 and 50 dB SPL. Above 4 kHz thresholds rise sharply; at 10 kHz thresholds are about 70 dB SPL. Thresholds also rise below 1 kHz, to about 65 dB SPL at 125 Hz.

In another laboratory, work on cellular mechanisms of transduction is being performed on the alligator lizard in order to relate the mechanical properties of the endorgan, the physiology of receptor cells and the discharge characteristics of the afferent neurons. These investigators hope to exploit the advantages of this preparation to gain insights into general mechanisms of transduction in hair cell systems including the mammalian cochlea. Investigations of the cochlear duct in the lizard have shown progress. The investigators have demonstrated that intracellular recordings can be made from single hair cells and supporting cells of the basilar papilla. Accounts of such cellular responses to acoustic clicks have been presented and a description of the potentials (including resting and endocochlear potentials) is being prepared. A supplementary ultrastructural study shows the presence of low-resistance gap inductions between hair cells and supporting cells, as well as between supporting cells; this suggests that significant electrical coupling may exist in the receptor. Additionally, preliminary data have been obtained concerning motion of the basilar membrane with the Mossbauer technique. Further studies appear to be logical and could be accomplished with established techniques. The roles of sensori-neural and mechanical factors in shaping tuning curves of fibers in the auditory nerve seem particularly promising. Additional data on displacement of the basilar membrane will allow correlation with data on unit responses in regionally associated hair cells and their nerve fibers. Information from these three levels has not been successfully recorded in any other animal and it might well contribute to a more definite understanding of the basic mechanism of transduction in the basilar papilla. The preparation may also be useful in testing existing models of cochlear transduction, since sound-induced impedance changes might be tested in the hair cells by examining changes in intracellular responses produced by passing electrical current through the cell membrane via microelectrodes. The peculiarities of the polarization pattern of the hair cells and associated differences in innervation and technical relationships would provide a sound anatomical basis for the suggested studies of two-tone rate suppression and the relationship between innervation and polarization of hair cells.

Another research group is currently directing its effort at developing a quantitative description of the neural encoding processes used by superior olivary complex neurons in processing auditory information. It is the basic premise of this project that the encoding processes can only be described in terms of the relationship between stimulus parameters and the distribution of neural activity over time and space within the population of activated neurons. Single units within the complex are sampled and the data obtained are used to reconstruct the population distribution over time and space in the form of neurogram. Time is represented by the post-stimulus onset time, space by the unit's characteristic frequency and discharge in terms of instantaneous rate. To minimize the problems of obtaining a sufficient sample size only SOC units located lateral to the medial superior olive (MSO) are being studied in detail. Results consist of an atlas of laterally-located superior olivary complex (SOC) groups which was developed to aid in classifying units into cell groups and to aid in determining the tonotopic organization of the cell groups. The atlas was required because cell group configuration varied in the rostrocaudal plane which led to difficulties in localizing units on a standardized figure. Two atlases were developed, one for frozen tissues, which suffered little shrinkage in the histological procedures used and one for celloiden-embedded tissues, which were reduced 50 percent in volume during dehydration. The atlases consisted of drawings outlining the boundaries of cresyl-echt-violet stained cell groups. The cell group membership of 441 units was determined and the location of 197 units outside the lateral superior olive (LSO) were indicated on the atlas drawings to examine the tonotopic organization of these structures. Units located within the caudal margin of the LSO (pLSO units) had a tonotopic organization similar to that of LSO units. Units located dorsal and ventral to the LSO were organized such that low frequency sensitive units were located laterally and high frequency sensitive units were located medially. Detailed maps indicating shifts in the rostrocaudal plane are included in a paper submitted to the Journal of Comparative Neurology.

Another team of investigators is engaged in the development of a system for fully automated tonal threshold audiometry in which the middle components of the auditory evoked response (AER) serve as response indices. The system is to be validated on subjects with normal hearing and on subjects with known disorders of communication. The clinical applicability of the automated system is to be assessed on actual clinical populations. Computer programs were developed to control fundamental frequency, intensity level, and repetition rate of the tonal stimuli. A study on the relations between rise-time, duration and AER magnitude and clarity confirmed the desirability of using tonal stimuli with short rise-times and no plateau duration. The spectrum of the 6 msec tone-pips (3 msec rise-fall time) they have selected is relatively narrow and appears clinically valid. A study on the effect of contralateral masking showed that the middle AER's seem to parallel voluntary behavioral responses in situations closely simulating clinical masking conditions. In another study, the effect of backward masking on the middle components is also being investigated. These procedures may be useful for the study of middle component AER to tone pips in neonates.

Cochlea or Inner Ear

In a series of studies that may lead to therapeutic procedures for controlling the peripheral microcirculation in the inner ear in many forms of deafness resulting from an inadequate supply of blood, a research team has been looking into the control of inner ear microcirculation. They attempted to determine whether or not the capillaries of the osseous spiral lamina and basilar membrane constrict or dilate under various conditions. Using an image splitter to place the split picture of a capillary on the CCTV screen the animal was made anoxic, stimulated by loud sounds or allowed to die. Dilation or constriction of the vessel was determined by overlapping or separating of the split image. No changes in vessel diameter were noted although marked changes in flow rate did occur. Indications are that the flow of blood in these terminal capillaries is determined by more central vessels, presumably those in the modiolus.

In the guinea pig, during general hypoxia produced by shutoff of respiratory air, oxygen-sensitive microelectrodes detect a decrease in oxygen concentration in the fluids of the tunnel of Corti before detecting a decrease in scala media oxygen concentration. The present experiments were designed to measure the cochlear microphonic (CM) potential generated by the organ of Corti when vibrated by a microprobe on the basilar membrane along with the oxygen decline in both tunnel and scala media to see upon which source of oxygen CM is dependent. Because oxygen concentration in both areas can decrease considerably before CM is affected, the recovery following a brief period of hypoxia is a more accurate measure. Because CM starts a recovery before scala media oxygen, the positive endolymphatic potential (EP) was also measured to determine its role in the generation of CM. The investigator's interpretation of the course of events is that CM is partially dependent upon oxygen supplied to the extracellular spaces of the organ of Corti by the spiral vessels and upon EP that, itself, is dependent upon several factors. The data indicate the EP plays a more complex role than that of providing a current flow for modulation by a resistance varying with vibration.

Another laboratory is investigating the structure and function of the inner ear. The rapid Golgi method was used on 18 newborn guinea pigs; cochleas from other newborn animals were stained for acetylcholinesterase, by the Maillet method, and examined by electron microscopy (TEM). The Golgi stained fibers were studied: (1) Nerve fibers in the spiral lamina which gave branches to capillaries, or terminated at the habenula were probably the previously described adrenergic fibers. (2) Many cochlear nerve fibers in the basal turn were studied. Their branches were limited to approximately the terminal 0.1 mm of each fiber, and generally supplied a single row of outer hair cells. (3) Efferent nerve fibers, which supplied outer hair cells, often coursed in the inner spiral bundle for some distance, thus having the possibility for influencing information coming from both inner and outer sensory cells. Data from chinchilla cochleas have been analyzed pertaining to the distribution of the crossed olivo-cochlear efferents. Results indicate that: (1) Some crossed efferent fibers travel in the inner spiral bundle for some distance; (2) the crossed efferents terminate on the outer hair cells and form axo-dendritic synapses in the Deiters cell region below; and (3) the contributions of the crossed efferents to the outer hair cells is not equivalent throughout the cochlea, but decreases apicalward.

A team of investigators is attempting in an ultrastructural study of the inner ear to advance our knowledge of the peripheral auditory nerves and understand Meniere's disease by morphologic studies of both human and experimental animals. In the study on the efferent nerve fibers, the crossed tract of the olivo-cochlear bundle was cut at the floor of the fourth ventricle in 11 guinea pigs. The animals were killed two to eight months later and perfused with Karnovsky's fixative. The nerve count was made at the vestibulo-cochlear anastomosis (bundle of Oort). The results indicate that 76 percent of the myelinated fibers of the vestibulo-cochlear anastomosis originate on the contralateral side and 24 percent on the homolateral side. However, it is interesting to find that the population of unmyelinated fibers did not change in the three specimens counted to date. In a previous study of, short survival time they found definite morphological changes in the unmyelinated fibers after midline nerve transection. Whether these fibers regenerated or other nerve fibers branched has yet to be determined. It is not certain which of the myelinated or unmyelinated fibers or both enter into the organ of Corti, or whether the unmyelinated fibers eventually approximate the blood vessels of the modiolus or if they abut the afferent nerve fibers.

In the nerve fiber study of the human organ of Corti, the population count was made at the habenula perforata and at Corti's tunnel. To date, two specimens (ages 7 and 31) have been examined along their cochlear lengths. Each segment was made identifiable on the millimeter scale of the cochlear spiral graph. In the 31-year-old specimen, nerve density was about 360 to 770 per mm in the basal turn (3 to 13 mm), but increased to 1200 to 1300 in the upper turns (18 to 22 mm) and then decreased to 630 at the apex. Twelve to 33 percent of these fibers passed through Corti's tunnel toward the outer hair cells. About 50 percent of these tunnel fibers passed through the fluid space while the other 50 percent lay on the floor of the pillar cells or were surrounded by these cells. In the 7-year-old specimen, the density at the basal turn (6 to 8 mm area) and at the upper turn (21 mm area) is similar, about 1270 per mm. In Corti's tunnel 17 to 20 percent of the fibers passed toward the outer hair cells. Again, approximately 50 percent of the tunnel fibers either lay on the floor or were surrounded by pillar cells. The above figures indicate that the nerve density at the habenula is low compared to the cat or squirrel monkey. The nerve population going toward the outer hair cells in humans is similar to that of the squirrel monkey (12 to 30 percent). The implication is that a majority of the nerve fibers go to the inner hair cells in humans as in other species.

One laboratory is attempting the quantification and identification of origin of the various summing potentials (SP) components and a delineation of the cochlear generator potential (GP). Several important results were obtained pertaining to SP and GP. These investigators succeeded in recording cochlear potentials from regions of the inner ear where inner hair cells (IHC) have been destroyed and where some outer hair cells (OHC) remained in apparently good condition (light microscopic criteria). These are the first experiments where recordings have become available from the pure OHC population. The converse, measures from pure IHC populations is relatively easily achieved. On the basis of the available IHC recordings they hypothesized that at moderate sound levels the OHC's produce much of the recorded SP, both positive and negative DIF SP components. This suggestion was made because when recording from IHC's alone it was observed that DIF-SP was significantly

reduced and DIF+SP altogether eliminated. It remained to be shown that in the absence of IHC normal SP is recordable. They now have two animals (guinea pigs) in which the normal SP was obtained from the fourth turn of the cochlear where the IHC's have been destroyed by heavy doses of kanamycin. These results support their contentions. They have numerous animals, however, in which appropriate damage patterns were accompanied by depressed cochlear potentials. Clearly light microscopy cannot reveal subtle morphological changes that might affect hair cell function. They intend to pursue correlation between patterns of cell destruction and cochlear potentials. Meanwhile, these preliminary findings support their general scheme of hair cell function.

Disorders of Hearing

One investigator is studying pathogenesis and bone resorption in aural cholesteatoma. This project is designed to study the mechanism of epidermal cyst formation and to elucidate the process of associated connective tissue breakdown. They studied the properties and differences of guinea pig and human collagenase and tested immuno-specificity of the anti-sera made against these enzymes. They are proposing to localize the collagenase in the tissue and cell of origin with an immuno-cyto-chemical method.

Guinea pig collagenase isolated from culture media of skin was separated into two enzymatically active fractions and then purified extensively. The immuno-specificity of their anti-human skin collagenase anti-serum was tested by immuno-diffusion and immuno-electrophoresis. Besides the strong band of identity they found a small second band. This did not occur when their anti-serum was reacted against partially purified or purified human skin collagenase. Further experiments on induction of cholesteatoma in guinea pigs have been carried out on 55 animals. The groups include free grafts of canal skin on the cochlea with or without talc, canal skin flaps on the cochlea with or without talc, talc on the cochlear or tympanic membrane and canal skin flap in a pocket under bulla mucosa. Epidermal cysts were found at the cochlea in 8 of 55 animals. Cochlea fistulas were found in 6 of 55 animals. The fistulas were associated with epidermal cysts in 3 cases, otitis media in 2 cases and talc granuloma without epidermal cysts in one case. The canal skin flap under bulla mucosa with a 30 percent incidence of epidermal cysts and a 40 percent incidence of cochlea fistulas appears to be the most reliable method of inducing bone resorbing cholesteatomas.

Another investigative team has as its objective to determine the mechanisms involved in serous otitis media in humans and to find a suitable animal experimental model to test a proposed hypothesis. The hypotheses are: (1) Initial infection starts the metaplastic changes of the mucosa, and this stimulated mucosa secretes enzyme(s) and immunoglobulins which inhibit bacterial growth causing (OME) otitis media with effusions; or (2) the initial infection may sensitize the middle ear mucosa and tubal epithelium and subsequent infection may result in middle ear effusion by either immediate or delayed hypersensitivity. In this concept, after initial sensitization even dead bacteria or bacterial components can elicit the hypersensitivity reaction. The aims were: (1) To establish correlation between levels of lysozyme and immunoglobulins and success rate of bacterial culture in the effusions; (2) clarify the role of mucosal secretion in the formation of effusions; (3) to devise practical diagnostic laboratory procedures if the above correlation is

A team of investigators is attempting in an ultrastructural study of the inner ear to advance our knowledge of the peripheral auditory nerves and understand Meniere's disease by morphologic studies of both human and experimental animals. In the study on the efferent nerve fibers, the crossed tract of the olivo-cochlear bundle was cut at the floor of the fourth ventricle in 11 guinea pigs. The animals were killed two to eight months later and perfused with Karnovsky's fixative. The nerve count was made at the vestibulo-cochlear anastomosis (bundle of Oort). The results indicate that 76 percent of the myelinated fibers of the vestibulo-cochlear anastomosis originate on the contralateral side and 24 percent on the homolateral side. However, it is interesting to find that the population of unmyelinated fibers did not change in the three specimens counted to date. In a previous study of short survival time they found definite morphological changes in the unmyelinated fibers after midline nerve transection. Whether these fibers regenerated or other nerve fibers branched has yet to be determined. It is not certain which of the myelinated or unmyelinated fibers or both enter into the organ of Corti, or whether the unmyelinated fibers eventually approximate the blood vessels of the modiolus or if they abut the afferent nerve fibers.

In the nerve fiber study of the human organ of Corti, the population count was made at the habenula perforata and at Corti's tunnel. To date, two specimens (ages 7 and 31) have been examined along their cochlear lengths. Each segment was made identifiable on the millimeter scale of the cochlear spiral graph. In the 31-year-old specimen, nerve density was about 360 to 770 per mm in the basal turn (3 to 13 mm), but increased to 1200 to 1300 in the upper turns (18 to 22 mm) and then decreased to 630 at the apex. Twelve to 33 percent of these fibers passed through Corti's tunnel toward the outer hair cells. About 50 percent of these tunnel fibers passed through the fluid space while the other 50 percent lay on the floor of the pillar cells or were surrounded by these cells. In the 7-year-old specimen, the density at the basal turn (6 to 8 mm area) and at the upper turn (21 mm area) is similar, about 1270 per mm. In Corti's tunnel 17 to 20 percent of the fibers passed toward the outer hair cells. Again, approximately 50 percent of the tunnel fibers either lay on the floor or were surrounded by pillar cells. The above figures indicate that the nerve density at the habenula is low compared to the cat or squirrel monkey. The nerve population going toward the outer hair cells in humans is similar to that of the squirrel monkey (12 to 30 percent). The implication is that a majority of the nerve fibers go to the inner hair cells in humans as in other species.

One laboratory is attempting the quantification and identification of origin of the various summing potentials (SP) components and a delineation of the cochlear generator potential (GP). Several important results were obtained pertaining to SP and GP. These investigators succeeded in recording cochlear potentials from regions of the inner ear where inner hair cells (IHC) have been destroyed and where some outer hair cells (OHC) remained in apparently good condition (light microscopic criteria). These are the first experiments where recordings have become available from the pure OHC population. The converse, measures from pure IHC populations is relatively easily achieved. On the basis of the available IHC recordings they hypothesized that at moderate sound levels the OHC's produce much of the recorded SP, both positive and negative DIF SP components. This suggestion was made because when recording from IHC's alone it was observed that DIF-SP was significantly

reduced and DIF+SP altogether eliminated. It remained to be shown that in the absence of IHC normal SP is recordable. They now have two animals (guinea pigs) in which the normal SP was obtained from the fourth turn of the cochlear where the IHC's have been destroyed by heavy doses of kanamycin. These results support their contentions. They have numerous animals, however, in which appropriate damage patterns were accompanied by depressed cochlear potentials. Clearly light microscopy cannot reveal subtle morphological changes that might affect hair cell function. They intend to pursue correlation between patterns of cell destruction and cochlear potentials. Meanwhile, these preliminary findings support their general scheme of hair cell function.

Disorders of Hearing

One investigator is studying pathogenesis and bone resorption in aural cholesteatoma. This project is designed to study the mechanism of epidermal cyst formation and to elucidate the process of associated connective tissue breakdown. They studied the properties and differences of guinea pig and human collagenase and tested immuno-specificity of the anti-sera made against these enzymes. They are proposing to localize the collagenase in the tissue and cell of origin with an immuno-cyto-chemical method.

Guinea pig collagenase isolated from culture media of skin was separated into two enzymatically active fractions and then purified extensively. The immuno-specificity of their anti-human skin collagenase anti-serum was tested by immuno-diffusion and immuno-electrophoresis. Besides the strong band of identity they found a small second band. This did not occur when their anti-serum was reacted against partially purified or purified human skin collagenase. Further experiments on induction of cholesteatoma in guinea pigs have been carried out on 55 animals. The groups include free grafts of canal skin on the cochlea with or without talc, canal skin flaps on the cochlea with or without talc, talc on the cochlear or tympanic membrane and canal skin flap in a pocket under bulla mucosa. Epidermal cysts were found at the cochlea in 8 of 55 animals. Cochlea fistulas were found in 6 of 55 animals. The fistulas were associated with epidermal cysts in 3 cases, otitis media in 2 cases and talc granuloma without epidermal cysts in one case. The canal skin flap under bulla mucosa with a 30 percent incidence of epidermal cysts and a 40 percent incidence of cochlea fistulas appears to be the most reliable method of inducing bone resorbing cholesteatomas.

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established; (4) to see if mucosal metaplasia leads to cholesteatoma; and (5) to determine if surface-active substances are involved in OME. The results were 190 middle ear effusions from 188 patients who were diagnosed as having OME. They also obtained 170 mucosal biopsies. Patients' ages ranged between one and twelve years.

In order to see if immunoglobulins and bacteriocidal enzymes are present in middle ear effusions, and to see if any correlation exists between recovery of microorganisms in relation to the concentration of immunoglobulins and bacterial enzymes, the specimens were examined for immunochemistry, microbiology, enzymological and ultrastructural investigation. (a) The middle ear pellets obtained by centrifugation of the middle ear effusions contained microorganisms in over 77 percent of the specimens. Many of the organisms showed varying stages of calcification in an ultrastructural investigation. However, only 50 percent of all specimens examined were culture-positive for the microorganisms. The commonest organisms were *H. influenzae*, followed by *Staph. epidermidis*, *Diphtheroid*, *Group A Streptococcus*. (b) Specific immunoglobulins that were examined include IgA, IgG, IgM and IgE, and the bacteriocidal enzyme was lysozyme. There was good evidence that immunoglobulins A & G are locally produced and their production increased with age. Immunoglobulin A in particular reflects rapid production up to age two, and slowly increased to age eight where their line cut-off was. In the case of immunoglobulin G, it has a steady increase above the serum level up to age eight. The level of IgM in the effusions, on the other hand, was lower than that of sera, and the level of IgM in effusions on an age scale was parallel to that of the sera.

The lysozyme level was dramatically increased in the effusion compared to the corresponding sera. The lysozyme level also increased with age, like IgA and IgG. In some cases in the older age group, the lysozyme levels in the effusions were often three hundred times larger than those of the sera. On the other hand, the percentage of bacterial positive cultures from OME patients showed a decreased rate of positive recovery with increase of age.

In their animal model ten squirrel monkeys have been inoculated with *D. pneumoniae* for the purpose of causing acute otitis media and then the immunologic changes in the middle ear as well as in sera were examined to see if the middle ear reacted following the infection. One important finding is that the middle ear has its own local defense system which matures with age. The important immunoglobulins involved in the immunodefense system are secretory IgA, IgE and IgG in chronic otitis media with effusions.

Acoustic Prosthesis

A group of investigators are attempting to develop an acoustic prosthesis. The object is to determine whether it is possible to excite a series of restricted sectors of the acoustic nerve from within the cochlea. This information is necessary for the development of a greatly improved acoustic nerve stimulation prosthesis that could potentially provide information sufficient for the direct hearing of intelligible speech for a significant population of the profoundly deaf. The aims were: (1) To determine the degree to which discrete electrical stimulation of predetermined restricted

sectors of the acoustic nerve can be effected with bipolar stimulation of the nerve from within the scala tympani; (2) to map the sensitivity of response of primary auditory neurons across the array of nerve fibers distributed within the bony spiral lamina, as a function of cochlear place, for a series of bipolar and multichannel intracochlear stimulating electrodes; (3) to derive quantitative threshold data from isolated colliculus neurons driven by electrical and sound stimuli, for determination of design parameters necessary for construction of the electronic system that must be used to drive any multichannel electrode; (4) to determine the histopathological consequences of implantation of electrodes within the scala tympani in prior-normal and prior-deafened animals; (5) to determine the histopathological consequences, if any, of long-term heavy stimulation of the acoustic nerve; and (6) to systematically evaluate the functional viability of primary auditory neurons surviving long-term cochlear implantation and stimulation. Sixteen neurophysiological studies evaluating implanted multichannel arrays in cats have been conducted. Seven other cats are now implanted with multichannel electrodes and await physiological and histopathological examination. Single unit studies have revealed that it is possible to excite a series of restricted sectors of the acoustic nerve array from within the scala tympani with appropriately positioned electrodes mounted in silastic carriers that fill the scala (displace perilymph). The effective displacement of perilymph by the electrode (and the connective tissue that grows around it) appears to be essential for achieving restricted excitation of the nerve. A stimulator stimulating the output of the multichannel electrode driving systems under construction has been used in these studies; critical design parameters for this system have been established in these experiments and have reflexly established that the design parameters are very reasonable. In development of better intracochlear stimulating electrodes, studies were directed toward problems inherent in implanting flexible electrodes into the middle cochlear turn in animals and man. Successful implantation of these long electrodes has been achieved with electrodes with an appropriately constructed central stiffening rib. These electrodes can, then, be safely implanted into the middle cochlear turn. Second, studies were directed toward development of a new fabrication procedure for producing scala tympani implant arrays. A technique for obtaining precise soft metal casts of the scala tympani has been developed, and a number of these casts have been made for the cochleas of guinea pigs, cats and man. Methods for construction of a precision die from these soft metal casts, from which dimensionally superior electrode arrays can be produced, have been developed. The generation of these better dies is essential to the efficient determination of critical electrode nerve interface parameters.

Balance

A group of researchers are engaged in a study of recorded body sway in order to evaluate equilibrium. The ultimate objective is to develop the technique of recording the body sway of the standing human subject into a clinically useful procedure. The research focuses on two clinical tests: (1) The quantitative Romberg test; and (2) the galvanic body-sway test. The overall goals were to be achieved by: (1) Developing instrumentation and techniques for administering the tests and quantifying the results; (2) systematically studying the characteristics of normal and abnormal standing

body sway and galvanic response; and (3) determining the best procedures for administering and quantifying the tests. Thusfar, in meeting the first goal they have: (a) Constructed a platform with quantitatively controllable stability; (b) incorporated an analog tape recorder into the recording system; and (c) completed a computer programming to obtain means, variances and frequency spectrograms from the recorded data.

The platform constructed balances on a central pivot whose stability is controlled by variable-tension-springs mounted at each corner. However, it became apparent that this design would not accomplish the intended purpose because the stability of the platform varied according to its resting position (i.e., if the person standing on it forced it into a tilted position, the resultant increased stretch of the springs on one side would increase the platform's stability). Therefore, they are now constructing an unstable platform of another design utilizing hydraulic cylinders at each corner. Fluid flow through the cylinders is controlled by a regulating valve. In the study of galvanic body sway in normal and abnormal subjects, the subject stood with feet together, shoes on, and eyes closed. Current was applied to the mastoid area with an aluminum foil electrode. The stimulus was generated by a constant-current generator with selectable rise time, fall time, duration and amplitude. Galvanic response amplitudes relative to prestimulus baseline were measured in two ways: (1) Maximum sway in the correct direction during current application ("maximum amplitude"); and (2) amplitude at a point in time after current onset where the maximum responses occurred most frequently ("fixed-latency amplitude"). Five each of the four possible responses (right ear +, left ear +, right ear -, left ear -) were obtained and averaged. Galvanic unilateral weakness (difference between ears) and directional preponderance (difference between right sway and left sway) were obtained. The results of the variability study provide part of the information necessary to arrive at the optimal clinical galvanic procedure. Best results were obtained when percent of unilateral weakness was obtained with fixed latency amplitudes (only 22 percent false-negative). Effort is proceeding to further reduce this incidence by using the fact that the galvanic test is used usually to determine whether a caloric deficit is of nerve or end-organ origin. By considering significant only galvanic deficits on the same side as caloric deficits, half of the potential false-positives are eliminated, and the "single-tail" limit of normal yields a false-negative incidence of only 12 percent.

Another team of investigators studying the physiology of the vestibular system in squirrel monkeys is attempting to elucidate the function of the various endorgans of the peripheral vestibular apparatus and of the ways in which information encoded peripherally is transformed to central pathways, more particularly, the vestibular nuclei. More specifically, the goal was to determine the discharge characteristics of peripheral otolith afferents and to continue studies of the functional organization of the vestibular nuclei.

With regard to the otolith organs, results showed: (1) Both the saccular and utricular maculae function mainly as equilibrium organs: (2) the two organs respond only to shearing displacements of the otolithic membrane. Compressional forces are ineffective when presented alone and do not modify

the response to simultaneously presented shearing forces. (3) Centrifugal force was used to determine input-output functions in the ± 5 g range. On this basis, it was concluded that the response in the presumed physiological (± 1 g) range is determined by three factors, tentatively identified as a receptor bias, a transduction gain, and a mechanical gain. (4) Response dynamics were characterized. Regularly discharging neurons have tonic discharge properties with their response paralleling the applied force profile. Irregular neurons were more phasic. In the study of the superior vestibular nucleus (SVN) it was shown physiologically that the great majority of SVN neurons receive bilateral inputs from parallel canals, the ipsilateral canal providing the monosynaptic excitatory input. Most units influenced by stimulation of the ipsilateral superior canal were located in the lateral SVN; posterior-canal neurons were found more medially. Relatively few horizontal-canal units were encountered. The work on otolith neurons, coupled with their previous studies of canal neurons, provides a comprehensive picture of the functional properties of all five vestibular endorgans in the primate and, as such, should be of help in disentangling the complicated physiology of the central vestibular pathways and in the clinical diagnosis of peripheral vestibular disorders.

Another laboratory involved in studies of vestibular mechanisms has as the overall objective the quantification of the functional connection during production of nystagmus between the receptor organs of the labyrinth (cristae and maculae) and neck proprioceptors and the oculomotor control system. Results have shown that it is possible to monitor the neural responses associated with the production of nystagmus in the abducens nerve, even during rotations of the head with large velocities, thus providing the opportunity to obtain estimates of correlation between firing of the motoneurons and the resulting eye movements. Data has been collected on 40 nerve fibers during the production of spontaneous eye movements and optokinetic and vestibular nystagmus. Results are consistent with the hypothesis that the changes in motoneuron firing for all types of eye movements are linearly related to both eye position and velocity. During vestibular nystagmus their change in frequency of firing exhibits the same mathematical relationship to the change in the eye position independently of the type of eye movement, agonistic or antagonistic. Differences in the neural responses were found, however, between vestibular and optokinetic nystagmus. Analysis of neural responses associated with eye movements induced by high frequency head rotations suggests the existence of a delay in neuromuscular transmission. The effect of this delay is to create a phase difference between the neural response and the eye movement greater than the one predicted solely from the low-pass characteristic of the eye globe.

Speech and Language

An investigator is engaged in a project to restore the function of paralyzed symmetrical muscles of the larynx, pharynx, face and neck. Thusfar, he has: (1) Developed a recording and stimulating system; (2) constructed a device for stimulating paralyzed muscles so that they track the motion of symmetrical, normal muscles; (3) measured the contraction of laryngeal muscles in vivo; (4) determined the system parameters when the transducer output from normal muscles is used, through external equipment to control

the electrical stimulation of a paralyzed muscle; (5) developed transducers for measuring muscle activity and tested these transducers and electrodes in chronic dogs and is attempting to determine some of the features of innervation of normal and reinnervated laryngeal muscles. The muscle stimulating device is being tested on a series of dogs. A length transducer capable of measuring the shortening of an isotonic contraction is now being tested as well.

Another investigator is attempting to assess the effectiveness of a reed-fistula method of speech rehabilitation following total laryngectomy. The speech of a series of patients who have undergone pharyngolaryngectomy and who have been fitted with a reed-fistula appliance are being examined: (1) To measure speech intelligibility; and (2) to analyze fundamental frequency, phonation time and rate properties. The methods used to compare reed-fistula speech with other types of alaryngeal and normal speech are: perceptual ratings of speech acceptability, comparisons of physical characteristics and intelligibility characteristics.

A complete set of necessary recordings on four patients have been completed.

Another investigator is attempting to devise a means to detect laryngeal pathologies by means other than direct visual examination, and to develop methods of high speed processing of data obtained by indirect laryngoscopy high speed (5,000 frames per second) photography of the larynx. A system was installed which allows an operator to hand trace the outline of the glottal opening which is projected onto a drawing surface. This outline information is transferred to a minicomputer where the area is calculated, displayed to the operator and saved for later processing. The system processes data obtained from 90 reels of film measuring glottal areas, speed quotient, open quotient, opening phase, closing phase, closed phase, jitter and shimmer, on about 27,000 frames. This is one of the largest studies attempted in high speed laryngeal photography. Presently, operators are able to achieve 125-150 frames per hour for sustained periods (2-1/2 - 3 hours). Results indicate that the results agree with those obtained by the traditional technique of measurement with a planimeter, but with at least an order of magnitude increase in data processing speed.

In an investigation on lateral dominance and lateral word recognition, a group of researchers studied the question of dependence of right visual field (left hemisphere) superiority for speed of vocal report of language and non-language stimuli. The experiment found only non-significant right visual field superiorities obtained from vocal reports of dot-present or dot-absent stimuli. Another investigation utilizing single letter stimuli yielded substantial RVF superiorities for right handed subjects (mean = 37 msec.), a smaller but statistically significant RVF superiority by left handers (mean = 14 msec.) and a significant handedness by visual half-field interaction. Left handers without left handed relatives showed no RVF superiority. Results support the conclusion that discriminatory response was required for demonstrating left hemisphere processing speed superiority, simple transcallosal transfer time for unstructured stimuli was much shorter than Filbey and Gazzaniga had suggested, and that the letter report task could differentiate groups whose cerebral organizations are thought to differ. The group

studied adolescent males who clearly met the general criteria of dyslexia and who also appeared likely to be dyslexic into adulthood. A number of lateralizing tasks were given. The results provided clear evidence of left hemisphere language lateralization in both dyslexic and normal control subjects on the vocal reaction time task for letter, dichotic digits and bilateral word recognition. The complexity of the study precludes complete summarization, but the findings indicate that two distinct defects may be present in chronic dyslexia.

Taste

A group of investigators are studying the mechanisms of taste function including detailed studies of direct binding of quinine to bovine taste tissues. Quinine, which tastes bitter to humans and is rejected by cows in taste-tests, was used as a ligand in binding experiments with various types of preparation of taste and non-taste tissues from bovine tongue. Fluorescence assays were used to quantitate quinine binding, because the compound is strongly fluorescent. Studies were carried out using taste papillae in situ, those excised from the tongue, homogenates of entire papillae, homogenates of epidermis of circumvallate papillae, and isolated taste cell suspensions; in each experiment, comparable control tongue tissue devoid of taste buds were included. Binding of small quantities of quinine to tissue was readily determined (levels of 50 nanomoles/mg protein). In general, however, there was as much binding to the non-taste preparations as occurred to those which contain taste buds. This result is in contrast to earlier experiments involving other types of taste stimulus molecules for which specific binding could be demonstrated. Therefore, although binding of quinine to taste tissue can be quantitated, measurement per se is not a specific indicator of taste specificity. At the present level of analysis, however, the data do not exclude the possibilities either that specificity determination in quality recognition for bitter taste may lie subsequent to the initial binding of quinine, or that specificity may be a property of the relative orientation of the stimulus molecule at the receptor membrane. Further exploratory studies to label monellin radioactively and to use it to study localization of the taste receptor sites are being undertaken.

Olfaction

Another group of investigators studying the functional anatomy of olfactory and taste receptors have established that neurons differentiate from the basal cells of the olfactory neuroepithelium, that the maturation period from the last division of the neuroblast is in the range of 8 to 10 days and that the life span of each cell is in the range of some 30 to 35 days. They have also established, contrary to the belief of previous authors, that vomeronasal receptors do turnover in rats and mice. Their turnover rate is now being evaluated. The study of degeneration of the olfactory mucosa (as induced by section of the olfactory axons) and the following regeneration has been completed. The pattern of degeneration-regeneration which can be observed in the olfactory bulb glomeruli as a consequence of the peripheral degeneration-regeneration of the neurons has been studied and completed in the frog and is 75 percent complete in the rat and mouse.

An investigator studying the electrophysiology of olfactory discrimination previously obtained electrophysiological and gas chromatographic data from frogs which suggested that one of the mechanisms basic to olfactory discrimination may be a chromatographic-like process which differentially sorbs odorants across the mucosal sheet. That is, the molecules of different odorants may migrate across the mucosa at different rates so that in the time frame of a sniff they would be distributed in different space-time patterns. To test this hypothesis, two lines of investigation are being followed. First, they are mapping the distribution of radioactive odorants across and within the frog olfactory mucosa. Secondly, they have been recording electrophysiologically from single units in the olfactory bulb to determine whether these differential molecular distributions across the mucosa are reflected centrally. They determined that butanol and octane are very differently distributed across the mucosa in a quickly frozen frog after a controlled sniff of tritiated odorant. This bears out their chromatographic-like process.

Touch

In an investigation of cutaneous coding and pattern perception a team of researchers are exploring the variables which affect vibrotactile pattern perception. To achieve this objective, the project is involved in two series of studies; one concerned with complex patterns such as might be generated by letters of the alphabet and a second area concerned with simple patterns which can be studied psychophysically. The project is particularly concerned with the effects of spatial manipulations on both complex and simple patterns. Tests were performed on blind, experienced users of the Optacon on some pattern identification tasks. To see the extent previous visual experience would aid tactile pattern recognition, a series of 15 letter-like patterns were designed which the subjects did not see until the end of the experiment. The subjects were presented with the patterns tactually on the abdomen via a 10 X 10 array of vibrators of the Kinotact. The trial-by-trial feedback was given after approximately 360 trials and the subjects attempted to draw the patterns. It was hypothesized that if a significant transfer from visual to tactile pattern recognition exists, the subjects should be able to identify those patterns they were familar with visually (letters) more than unfamiliar patterns (non-letters). The results did not support a transfer hypothesis. Subjects were unable to identify letters more readily than non-letters. Subjects were unable to assign correct numbers to the letter patterns with a greater frequency than the non-letter patterns. Only one of the five subjects could draw any of the patterns correctly. Results do not support the hypothesis that there is a strong visual imagery associated with tactile pattern perception.

CONTRACT NARRATIVE
Communicative Disorders Program, NINCDS
Fiscal Year 1976

JOHNS HOPKINS UNIVERSITY (N01-NS-2-2318)

Title: Study of Cutaneous and Visual Patterned Stimulation Communication Aids for the Profoundly Deaf Infant

Contractor's Project Director: Moise H. Goldstein, Ph.D.

Current Annual Level of Support: Extended without funds.

Objectives: The original objective was to develop techniques and instrumentation for studying and evaluating the feasibility of utilizing cutaneous or visual stimulation derived from a deaf infant's own vocalizations in order to provide information feedback and to increase the infant's vocalizations and speech. During the present contract year, an additional objective has been to obtain basic psychophysical information concerning tactile pattern discrimination in young children and adults.

Major Findings: Children in all three experimental groups (visual feedback, tactile feedback, and control group) showed increased vocalizations; substantial differences were not obtained among the three groups although more refined statistical analyses are continuing. Results of the psychophysical studies suggest that the cue of duration has particular salience for absolute identification of tactile stimuli.

Significance to NINCDS Program and Biomedical Research: The feasibility of tactile aids for the deaf deserves full exploration; this project is contributing useful information toward assessing suitability of tactile aids for very young children.

Cooperating Units: In previous years, this contract was monitored by the Biomedical Engineering Section.

Proposed Course of Contract: This contract will terminate December, 1976. The investigators may submit a grant application to continue specific aspects of the project. Last summer, NINCDS convened a workshop on Tactile and Visual Aids for the Deaf; two investigators from this contract participated in that workshop where they presented their results and provided future direction for this type of research.

CONTRACT NARRATIVE
Communicative Disorders Program, NINCDS
Fiscal Year 1976

VETERANS ADMINISTRATION HOSPITAL, MINNEAPOLIS, MINNESOTA (N01-NS-4-0019)

Title: Development of a Research Tool Concerning Speech and Language Therapy for Aphasic Adults

Contractor's Project Director: Robert H. Brookshire, Ph.D.

Current Annual Level of Support: \$84,648 (FY 75)

Objectives: The contractor will develop a descriptive and quantifiable system of coding the content of speech and language treatment sessions with aphasic adults. Coding results will differentiate between various types of therapeutic approaches on the basis of differences in treatment content. The system is needed as a tool for conducting research contrasting the efficacy of various therapeutic approaches for treatment of aphasia.

Major Findings: The contractor has developed an off-line system for coding the content of the therapeutic process with aphasic adults. During the pilot study, the system has been demonstrated to have the following capabilities:

- a) it records clinicians' behaviors, the tasks presented to patients, patients responses to clinicians' behaviors and tasks, and clinicians' responses to the patient behaviors,
- b) it quantifies events and describes the relationships between them,
- c) it has been demonstrated to differentiate among differing treatment sessions, such as those which are problem directed, involve language stimulation, melodic intonation therapy, etc., and
- d) it has been demonstrated to have an inter-coder reliability of greater than 0.85 following training by the project staff.

In addition, using the coding system the contractor has studied the effects of patient severity on the content of treatment and the degree of variability across and within clinicians. These results were recently presented at a scientific meeting.

Significance to NINCDS Program and Biomedical Research: This new procedure will provide a needed research tool. Not only will it quantify and differentiate among therapeutic approaches but also, it will provide a great deal of information on how variations in patient symptomatology, types of clinicians and settings, affect the treatment process.

Cooperating Units: None

Proposed Course of Contract: The contract will be extended to allow the contractor to meet the following new objectives:

- 1) To develop a short on-line coding system (on the basis of the item analyses of the long off-line system) for use in coding treatment content in real-time. Such a system would be less time consuming and could be used in settings where video tape is not available.
- 2) To evaluate the effectiveness of training coders in other settings with only a coding manual and training video tapes. It would be advantageous if the coding system could be used without direct training by the contract staff.
- 3) To conduct a full field evaluation of both the short on-line system and the long off-line system when used by persons in other settings. The evaluation will determine whether both systems are valid and reliable.

CONTRACT NARRATIVE
Communicative Disorders Program, NINCDS
Fiscal Year 1976

THE UNIVERSITY OF UTAH (N01-NS-4-2305)

Title: Development of a Manual for Impedance Testing in Children

Contractor's Project Director: Geary McCandless, Ph.D.

Current Annual Level of Support: No FY 75 funds
Terminated 7/27/75

Objectives: This manual provides a large amount of practical experience of the principal investigator and consultants in applying impedance measurement procedures to young children. The manual was written to enable audiologists who have not received extensive training in impedance procedures during their clinical training (widespread use of these techniques is relatively new) to develop expertise in the practical aspects of testing children. Research results and new procedures developed under several NINCDS grants have also been included.

Significance to NINCDS Program and Biomedical Research: This is a dissemination activity and will contribute to delivery of health services for children. The impedance test procedure is especially useful for detecting serous otitis media, a condition which usually produces a conductive hearing impairment while the condition persists. These test procedures, which can be quickly administered and which require no cooperation on the child's part, other than sitting reasonably still and refraining from talking or swallowing, can be utilized for large-scale screening activities in public schools, public health facilities, etc.

Cooperating Units: None

Course of Contract: The contract was terminated on schedule on July 27, 1975. A completed draft version of the manual was received in the fall of 1975. Program staff are presently editing the manual for publication purposes.

CONTRACT NARRATIVE
Communicative Disorders Program, NINCDS
Fiscal Year 1976

BOLT BERANEK AND NEWMAN (N01-NS-4-2322)

Title: Development and Testing of an Instrument for Assessing Speech Discrimination

Contractor's Project Coordinator: Daniel N. Kalikow, Ph.D.

Current Annual Level of Support: \$40,904 (FY 75)

Objectives: This contract was awarded in June, 1974, to develop a new test for measuring discrimination of connected speech where key words vary in predictability. This test is intended to measure the cognitive aspects of speech discrimination which are not assessed by presently available test instruments. In the second phase, beginning late in 1975, the contractor measured speech discrimination in the presence of a competing speech measure at various signal-to-noise levels for groups of subjects having normal hearing, and sensorineural hearing impairments to determine reliability of the test in a controlled clinical setting.

Major Findings: The contractor has completed development of the final form of the SPIN test which:

- 1) tests speech reception in different signal to babble noise ratios, and
- 2) contrasts subjects' abilities to receive speech in high and low probability contexts.

Experiments conducted on both normal and hearing-impaired subjects of various diagnostic categories have provided the following results:

- 1) eight of the forms are equivalent and can be used alternatively
- 2) hearing impaired subjects have reduced performance relative to normals on the SPIN test
- 3) the amount of reduction for the hearing impaired subjects is greater for low probability items, resulting in a greater gap between low and high probability curves for hearing impaired subjects than for normal subjects.

Significance to NINCDS Program and Biomedical Research: Available clinical tests are not highly successful at identifying, among persons with acquired sensorineural deafness, those individuals who are likely to experience improved receptive communication with an aid. To the extent that successful aid wearers utilize redundancy and predictability of speech messages to improve their understanding of speech, this test will improve prediction of successful hearing aid use.

Cooperating Units: Bolt Beranek and Newman, Inc. in Los Angeles and the

Massachusetts Eye and Ear Infirmary, Boston, Massachusetts.

Proposed Course of the Contract: Presently, the contract has been extended without additional funds. A proposal has been received from the contractor for conducting a field evaluation. The purpose is to determine the validity of the SPIN test as a predictive measure of the degree of benefit a hearing-impaired person will receive from a properly fitted hearing aid. This proposal is presently being reviewed for scientific merit. If the outcome is favorable, negotiations will be conducted for a one year extension with additional funds.

CONTRACT NARRATIVE
Communicative Disorders Program, NINCDS
Fiscal Year 1976

CITY UNIVERSITY OF NEW YORK GRADUATE CENTER (N01-NS-4-2323)

Title: Prescriptive Fitting of Wearable Master Hearing Aids

Contract Project Director: Richard E. C. White

Co-Principal Investigator: Harry Levitt

Current Annual Level of Support: \$198,116 (FY 74: 24 months funding) plus
\$ 10,525 (FY 75)

Objectives: The goal of this project is to determine the degree to which wearable master hearing aids (WMHA) used as prescriptive laboratory devices or modifiable training devices can enhance the final fitting of hearing aids and improve the communicative efficiency of the wearer. The special target population for this contract is middle aged persons with acquired sensorineural hearing loss who have not received benefit from conventional hearing aids.

Major Findings: The contractor has developed an experimental protocol for the prescriptive fitting of hearing aids. At different stages in development, this protocol was administered to two small groups of hearing impaired subjects. The results have been used to refine, select and delete some of the initially developed procedures. Recently, a final draft has been completed. This protocol, which will be evaluated during the second stage of the research, includes: a) a questionnaire, b) audiometric measures of sensitivity and speech reception, c) otoadmittance measures (tympanometry, static admittance, and reflex threshold), d) experimental tests of speech discrimination of nonsense syllables in noise and quiet, e) a convergence procedure including successive adjustments of the hearing aid parameters, and f) a final test session comparing performance with an aid selected clinically and the WMHA on tasks of speech discrimination, reception, and noise band thresholds.

Significance to NINCDS Program and Biomedical Research: Many hearing-impaired persons complain that hearing aids do not improve their hearing. This contract will develop procedures for prescribing for individual patients the most successful ensemble of acoustic characteristics available within the capabilities of present hearing aid design.

Cooperating Units: The wearable master hearing aids were developed under contract to the Biomedical Engineering program, C&FR.

Proposed Course of the Contract: The recently completed draft prescriptive protocol will be evaluated on a larger number of subjects having sensorineural impairments. During this phase the degree of communicative effectiveness obtained by the new fitting method will be compared with that obtained by conventional fitting methods. The outcome will be an effective and efficient protocol for the prescriptive fitting of hearing aids.

CONTRACT NARRATIVE
Communicative Disorders Program, NINCDS
Fiscal Year 1976

MASSACHUSETTS MENTAL HEALTH RESEARCH CORPORATION (NO1-NS-4-2324)

Title: Critical Age for Language Learning

Contractor's Project Director: Simeon Locke, M.D.

Current Annual Level of Support: Extended without additional funds

Objectives: The contractor was expected to complete two documents. One will be a complete critical review, analysis, and synthesis of currently existing biological and behavioral data pertaining to the hypothesis of a critical age or ages for human language learning. This critical review should be suitable for publication as a monograph or in a scientific journal. The contractor was also expected to outline research needed to achieve greater understanding of the process of human language learning and associated disorders as they relate to the critical age hypothesis.

Major Findings: In October, 1975, the contractor submitted the first two chapters of the critical review document. These were reviewed by outside advisors and program staff and found to be unsatisfactory. Not only did these chapters not review the necessary material, but also the writing would require considerable editing before it would be satisfactory for publication. The contractor was advised to proceed with writing the remaining chapters of the critical review document before revising those chapters already submitted.

In November, 1975, the contractor submitted the second document, outlining needed research. On review, it was evident that satisfactory development of a research directing document depended upon having a comprehensive and critical analysis of the information already available. Therefore, the contractor was advised to disregard the second document until the first document was complete.

Significance to NINCDS Program and Biomedical Research: There are large gaps in our understanding of the neurological bases of language and, as a result, there is limited understanding of many disorders of language development. Although behavioral scientists have occasionally shown interest in neuro-physiological research, the reverse has infrequently occurred. The synthesizing document is expected to stimulate interdisciplinary research on language development and to focus attention on research problems deserving special attention. After publication of the document, NINCDS expects to receive an increased number of high quality, investigator-generated grant proposals on topics in this general area.

Cooperating Units: A pediatric neurologist from the Perinatal Research Branch, NINCDS, has read and commented on some of the materials submitted by the contractor.

Proposed Course of the Contract: To allow the contractor time to complete the

final chapters of the critical review document, the contract was extended (without additional funding) to June 30, 1976. Additional chapters have been submitted and the contractor will complete the final chapters by the termination date. There are no plans to further develop the research document at this time.

The form of the document will be too large for publication as a journal article or monograph. Following termination of the contract, advisors will be consulted concerning whether the document, as a whole, merits editing for publication, or whether only certain sections should be considered for publication.

CONTRACT NARRATIVE
Communicative Disorders Program, NINCDS
Fiscal Year 1976

UNIVERSITY OF FLORIDA (N01-NS-5-2313)

Title: Study of Auditory Sensitivity and Discrimination in Young Children

Contractor's Project Director: William A. Yost, Ph.D.

Current Annual Level of Support: \$84,849 (FY 75)

Objectives: The goal of the project is to develop measuring techniques for assessing auditory sensitivity to pure tones and discrimination of complex auditory patterns in young children. Moreover, the relationship between sensitivity and discrimination would be correlated with the chronologic age of the subjects (0-6 yrs) and level of background noise during the test. The following sets of measures will be used: cardiac activity (ECG activity), auditory evoked potential, overt behavioral measures and non-nutritive sucking (conjugate reinforcement). Base-line data will be collected on normal subjects and finally, a test battery for measuring auditory sensitivity and discrimination in young children will be developed.

Major Findings: The contractor tested and evaluated the use of a Bekesy tracking procedure in conjunction with non-nutritive sucking using the conjugate reinforcement technique for testing auditory sensitivity. Contrary to published reports, this paradigm has not been found to occur in the infants tested above chance level. Therefore, this technique will be abandoned and a simpler method of using sucking as a behavioral indicator of auditory reception will be tried.

Pilot testing has confirmed:

- a) the usefulness of an adaptive psychophysical procedure for determining auditory thresholds in 3 to 5 year olds,
- b) that recording and processing evoked brain activity is feasible with adult subjects and must now be tried with young children, and
- c) that heart rate responses can be elicited with auditory stimulation in sleeping infants. Further work is needed to determine if these responses have sufficient clarity to allow thresholds to be determined.

Significance to NINCDS Program and Biomedical Research: Procedures are needed for assessing the hearing of young children. Without a battery of tests which are usable with all young children for assessing hearing acuity and speech discrimination, the degree of impairment in young children cannot be determined and appropriate treatment sought or evaluated. In addition, information is needed on whether there are changes in hearing functioning and responses to noise during development.

Cooperating Units: None

Proposed Course of the Contract: In the first phase of the contract, which will require 2 years, the major effort will be to refine and develop existing measures of auditory sensitivity and discrimination, particularly for use in very young subjects (newborns to 18 months of age).

The second phase involves three years of collecting base-line data for normal hearing children less than six years of age.

CONTRACT NARRATIVE
Communicative Disorders Program, NINCDS
Fiscal Year 1976

UNIVERSITY OF PITTSBURGH (N01-NS-5-2317)

Title: Study on Estimators of Aphasic Patients' Communicative Performance in Daily Life

Contractor's Project Director: Audrey L. Holland, Ph.D.

Current Annual Level of Support: \$87,586 (FY 75)

Objectives: The contractor will:

- a) develop a measure of communicative ability in daily life activities for use with aphasic adults,
- b) determine the validity of the new measure when compared to observer information and informant information on the communicative behavior of aphasic adults in their natural living situation,
- c) study the validity of presently available tests of aphasic language impairment as estimators of communicative ability and performance in daily life, and
- d) recommend methods for measuring the communicative ability of aphasic adults in daily life activities.

Major Findings: Following reiterative phases of development and revision, the contractor has completed a measure of Communicative Ability in Daily Living (CADL) and administered it to twenty normal or aphasic adults. This version is presently being pilot tested. Similarly, the contractor has developed an observation system for recording the number and type of communicative activities engaged in by aphasic adults as well as their degree of communicative success. Finally, an interview form for gathering information from families on patients' communicative performance in daily living has been developed.

Pilot studies are being conducted to determine the degree of difficulty of different daily life activities for aphasic adults on the CADL, from observation and family interviews.

Significance to NINCDS Program and Biomedical Research: A measure of the degree and type of communication handicap is needed for evaluating the efficacy of various types of treatment for aphasic adults. Such a measure will indicate the degree of dependence of such patients on others for meeting their daily needs. The types of measures to be developed for assessing the degree of communicative impairment in aphasic adults may be adapted to determine the degree of communicative handicap in adults with other types of hearing, speech and language disorders.

Cooperating Units: Veterans Administration Hospital, Boston, MA; Emerson

College, Boston, MA; Memphis State University, Memphis, TN; Mercy Hospital, Pittsburgh, PA; and the Veterans Administration Hospital, Pittsburgh, PA.

Proposed Course of Contract: Contingent upon successful completion of the pilot testing in June, 1976, studies of the validity and reliability of the new measure will be initiated and conducted over a one year period. Simultaneously, the validity of several of the existing measures of aphasic impairment for measuring communicative ability will also be determined. The contract will terminate in FY 1978.

CONTRACT NARRATIVE
Communicative Disorders Program, NINCDS
Fiscal Year 1976

THE REGENTS OF THE UNIVERSITY OF CALIFORNIA (N01-NS-5-2322)

Title: Measures of Children's Language Performance

Contractor's Project Director: Janice E. Laine, Ph.D.

Current Annual Level of Support: \$117,100 (FY 75 forward funded for 18 months)

Objectives: The objectives of this project include:

- a) developing a composite of measures of language performance in children which will assess small changes in the language performance of neurologically impaired children,
- b) preparing an administration and scoring manual which will train speech and language pathologists to be reliable examiners,
- c) demonstrating that the composite of measures is both valid and reliable for assessing small changes in the language performance of children delayed in language development.

Major Findings: A preliminary draft of a composite set of language performance measures was completed in January, 1976. Copies were sent to six persons with expertise in developmental psycholinguistics; three advisors to the NINCDS, and three to the contractor. All six reviews were favorable with some additions and deletions recommended. The measures have been revised and administered to a small number of language impaired children. Formal pilot testing will begin in the fall of 1976 following examiner training.

Significance to NINCDS Program and Biomedical Research: Measures are needed for assessing change in language performance in neurologically impaired children. Those instruments which are presently available are based on language development in normal children and are not sufficiently fine-grained to be sensitive to the small changes that occur in the language impaired populations. This new research tool will be used in future research to determine the process by which language impaired populations develop language and the similarities and differences from the normal process of language development. This information is needed for developing treatment methods for language impaired children. Also, the measures will be used for investigating the efficacy of various types of treatment for different groups of language impaired children.

Cooperating Units: Los Angeles County Schools.

Proposed Course of the Contract: The contractor will conduct a formal pilot test of the measures over the next 12 months to determine:

- a) intra- and inter-examiner reliability,

- b) which tasks can accurately identify children at particular linguistic levels, and
- c) which tasks or sets of tasks can discriminate between children with different proficiencies of language performance.

Contingent upon successful completion of the pilot testing phase, the contract will be extended for a full field evaluation of the new measures.

CONTRACT NARRATIVE
Communicative Disorders Program, NINCDS
Fiscal Year 1976

THE JOHN F. KENNEDY INSTITUTE (NO1-NS-5-2323)

Title: Study of Sensory and Perceptual Functioning of Young Children With and Without Delayed Language Development.

Contractor's Project Director: Rachel E. Stark, Ph.D.

Principal Investigator: Paula Tallal, Ph.D.

Current Annual Level of Support: \$245,169 (FY 75 forward funded to 1/30/79).

Objectives: The objective of this project is to conduct an experimental study of the performance of different types of impaired children on sensory, perceptual and cognitive tasks. The types of children to be studied include: language impaired, reading impaired, verbal dyspraxic and normal controls. The study will determine whether there are any direct relationships between patterns of task performance on the one hand, and children's primary problem, on the other hand. In addition, the study will determine whether a particular level of performance on any of the tasks is associated with a certain degree of impairment in language development, reading or speech articulation.

Major Findings: In the first six months, the contractor developed the final research plan including each of the experimental tasks for assessing sensory, perceptual and cognitive functioning in the auditory, visual and tactile modalities. In January, 1976, the contractor submitted the final plan which was reviewed by three advisors to the NINCDS as well as program staff. The plan was found to be satisfactory and the contractor was advised to proceed with examiner training and subject selection. Testing of normal controls and language impaired children will begin in May, 1976.

Significance to NINCDS Program and Biomedical Research: Many treatment programs of language impaired children have been based on the assumption that auditory processing difficulties are associated with impaired language development in many children. However, research results have been conflicting and have not always demonstrated that this is the case. There is need for a comprehensive study of the sensory, perceptual and cognitive abilities of each of these groups so that comparisons can be made across groups and with normal controls as well. If different performance characteristics in sensation, perception and cognition are found to be typical of different types of impaired children, such information will be directly relevant to improving the diagnosis, assessment and treatment of these children.

Cooperating Units: Howard County Public School System, MD; Dasher Green Co-operative Nursery, Columbia, MD; Chatworth School, Baltimore County, MD.

Proposed Course of the Contract The first phase of the contract will require 18 months for completion and include study of only the normal control group and

the language impaired subjects. Upon completion of the first phase, the contractor will submit a comprehensive report of the results. Outside advisors will be asked to review the documents and advise program staff concerning the merit of continuing the project to include study of reading impaired and verbal dyspraxic children on the exact same procedures.

CONTRACT NARRATIVE
Communicative Disorders Program, NINCDS
Fiscal Year 1976

SPECIAL SCHOOL DISTRICT OF ST. LOUIS (N01-NS-5-2324)

Title: Possible Effects of Hearing Aids on Auditory Sensitivity in Children

Contractor's Project Director: Robert L. Huskey

Current Annual Level of Support: \$79,758 (FY 75 funds--forward funded 30 months)
\$ 6,000 (FY 76 funds)

Objectives: This contract has the dual objectives of (1) determining whether hearing aids, as traditionally fitted to young children, produce significant amounts of temporary threshold shift and should such shifts be observed in any child, (2) determining optimal strategies for simultaneously minimizing observed threshold shifts while providing maximally useful auditory amplification.

Major Findings: The contractor has developed a protocol for measurement of relative temporary threshold shift. Only one of the five projected hearing loss groups (the moderate hearing loss group) has its quota of subjects. Data collection has begun for these subjects and preliminary results indicate little temporary threshold shift. It must be emphasized that the small amount of gain for the hearing aids used by this group does not allow generalization of these results to the more severely hearing-impaired groups. The contractor is currently emphasizing recruitment of severely hearing-impaired children in order to acquire a sample sufficient to permit generalization of the results.

Significance to NINCDS Program and Biomedical Research: There has been concern that habitual use of powerful hearing aids may cause permanent deterioration of hearing. This contract will empirically demonstrate the effects of hearing aids on auditory sensitivity for a sample large enough to permit generalization. This contract will have important implications for hearing aid evaluation and recommendation procedures.

Cooperating Units: None

Proposed Course of Contract: The contractor will recruit the remainder of the subjects by October, 1976, with special emphasis on the more severely hearing-impaired children. The bulk of the data will be collected during the next fiscal year, and detailed analyses of the data will begin and continue into the final contract period.

CONTRACT NARRATIVE
Communicative Disorders Program, NINCDS
Fiscal Year 1976

CINCINNATI PUBLIC SCHOOLS (N01-NS-5-2325)

Title: Study of Relations Between Children's Noise Exposure and Auditory, Language and Reading Skills

Contractor's Project Director: Joseph L. Felix, Ph.D.

Current Annual Level of Support: \$5,725 (FY 75, the contract was terminated four months after initiation)

Objectives: The objective of the project was to determine the relations, if any, between several measures of auditory performance, the noise levels of the children's environments over a continuing period of time, and speech, language and reading skills among children in the 10-12 age range.

Major Findings: On visit to the contractor during the contract negotiation phase, the program staff observed that the level of expertise of the contractor's staff who would be conducting the research did not seem congruent with the proposal which was submitted to the NINCDS. Therefore, a contract was funded for only four months and the contractor's staff asked to develop a data collection and analysis plan for the research. In August, the plan was submitted to the NINCDS and reviewed by two advisors and program staff. It was found to be unsatisfactory; it did not show an appreciation of the scientific and technical aspects of the problem as a whole and did not indicate that persons with expertise in psychoacoustics, noise, or audiology would be actively involved in all phases of the research. Continuation was not recommended and the contract was terminated in September, 1975.

Significance to NINCDS Program and Biomedical Research: At present, there is little available information concerning effects of noise on children's hearing. Adequate information is needed to answer each of the following issues:

- a) whether children who have lived for many years in a noisy environment demonstrate equal or poorer auditory sensitivity and discrimination as compared to children who have lived in a quiet environment,
- b) whether two such groups of children perform similarly when tested against a noise background, and
- c) whether such children's performances on psychoacoustic tasks are related to their speech, language or reading skills.

Cooperating Units: None

Proposed Course of the Contract: The Program is considering which support mechanism would be most effective in meeting the objectives of the intended research.

CONTRACT NARRATIVE
Communicative Disorders Program, NINCDS
Fiscal Year 1976

THE EYE AND EAR HOSPITAL OF PITTSBURGH (N01-NS-5-2331)

Title: Evaluation of Subjects Presently Fitted with Implanted Auditory Prostheses

Contractor's Project Director: Robert C. Bilger, Ph.D.

Current Annual Level of Support: \$76,604 (FY 75)
\$ 8,327 (FY 76)

Objectives: Subjects fitted with auditory implant prostheses prior to the initiation of the project, will be studied to determine their auditory sensitivity, auditory discrimination skills, speech perception, and vestibular functioning. Other pertinent information such as user satisfaction, lipreading skills, cognitive function, conceptualization, and emotional stability will also be gathered.

The resulting information on the performance of persons with implanted auditory prostheses will direct investigators on how to improve these prostheses and will indicate to practitioners the degree of benefit which patients may receive from implanted prostheses which are presently available.

Major Findings: All subject testing has been completed and the results drafted in a final report. However, the results will not be released until approved by the project consultants in May, 1976.

Significance to NINCDS Program and Biomedical Research: Many deaf persons are unable to benefit from conventional hearing aids and an implanted auditory prosthesis which stimulates the auditory nerve directly is a promising experimental technique for overcoming their deafness. However, good scientific data concerning the performance of the device are necessary if progress is to be made in the future development and use of such aids. This project will be the first objective evaluation of the performance of persons with an implanted auditory prosthesis.

Cooperating Units: None

Proposed Course of Contract: All subject testing has been completed. The final report will be distributed to the project consultants prior to a site visit in May, 1976. If the outcome of this review is favorable, the final report will be submitted for publication. Segments of the report will also be presented this year at several professional meetings.

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| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 NS 02102-03 CDP (Prev. Proj. No. Z01 NS 02102-02 C&FR) |
| PERIOD COVERED July 1, 1975 to June 30, 1976 | | |
| TITLE OF PROJECT (80 characters or less) Temporal and Masking Phenomena in Persons with Sensorineural Loss | | |
| NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT <div style="display: flex; justify-content: space-between;"> <div> PI: E.A. Cudahy OTHER: L.L. Elliott </div> <div> Fellow Northwestern Univ., Evanston, Ill. </div> <div> CD NINCDS </div> </div> | | |
| COOPERATING UNITS (if any) None | | |
| LAB/BRANCH Communicative Disorders Program | | |
| SECTION | | |
| INSTITUTE AND LOCATION NINCDS, NIH, Bethesda, Maryland 20014 | | |
| TOTAL MANYEARS: 1.5 | PROFESSIONAL: 1.0 | OTHER: 0.5 |
| SUMMARY OF WORK (200 words or less - underline keywords) Persons with a high frequency hearing loss frequently complain of difficulty understanding speech in noisy environments. A variety of tasks, using a 2IFC procedure, were employed to investigate <u>auditory temporal processing</u> in noise by listeners having either a <u>noise-induced</u> or <u>age-related hearing loss</u> . First, <u>temporal integration</u> functions were measured in wide-band noise for tonal durations ranging from 3 to 985 msec. at 500, 1500, 2000, and 4000 Hz. Second, detectability in the presence of a continuous narrow-band (400-800 Hz) noise masker was measured for 5- and 146-msec tonal signals at six frequencies from 500 to 4000 Hz. Finally, <u>forward</u> , <u>backward</u> , and <u>gap</u> masking were studied for two frequency combinations of a brief signal and a narrow-band noise masker. For gap masking, the masker was continuous except for a brief silence or "gap" of 20-, 50-, or 200-msec. duration during each observation interval. The results of these studies suggest poorer temporal resolution for these listeners than for the normal controls. Future work will further examine the temporal processing capabilities of persons with a high frequency hearing loss and will relate these results to speech reception by these listeners. | | |

PROJECT DESCRIPTION:

Objectives: Persons with a high frequency sensorineural hearing loss frequently complain of difficulty understanding speech in noisy environments. The present project is a continuation of parametric investigations of the auditory capabilities (with special reference to speech perception) of persons with hearing loss due to aging or to noise exposure. Initial work in this laboratory indicated that persons with sensorineural hearing loss have abnormalities in the temporal processing of acoustic signals in addition to their inability to detect sounds at normal intensity levels. The current studies focused on the perception of an auditory signal in the second or third formant region of speech with an interference stimulus in the first or second formant region.

Methods Employed: The procedures for the temporal integration, forward masking, and backward masking studies were reported in the previous Annual Report. During the current year, data collection in the backward and gap masking studies were initiated and the masker was changed to a narrow band noise. In the gap masking study, the noise was on continuously except for a brief silence or "gap" of 200, 50 or 20 msec. Detection was measured for a brief tonal signal presented at various time intervals relative to the beginning of the gap.

Subjects: Adult volunteers with pure tone audiograms indicating presbycusis or noise-induced hearing loss are employed through the Normal Volunteer Program. Two adult volunteers with normal pure tone audiograms participate as controls. Subjects are pledged to report to the laboratory for three test sessions per week over a minimum period of six months.

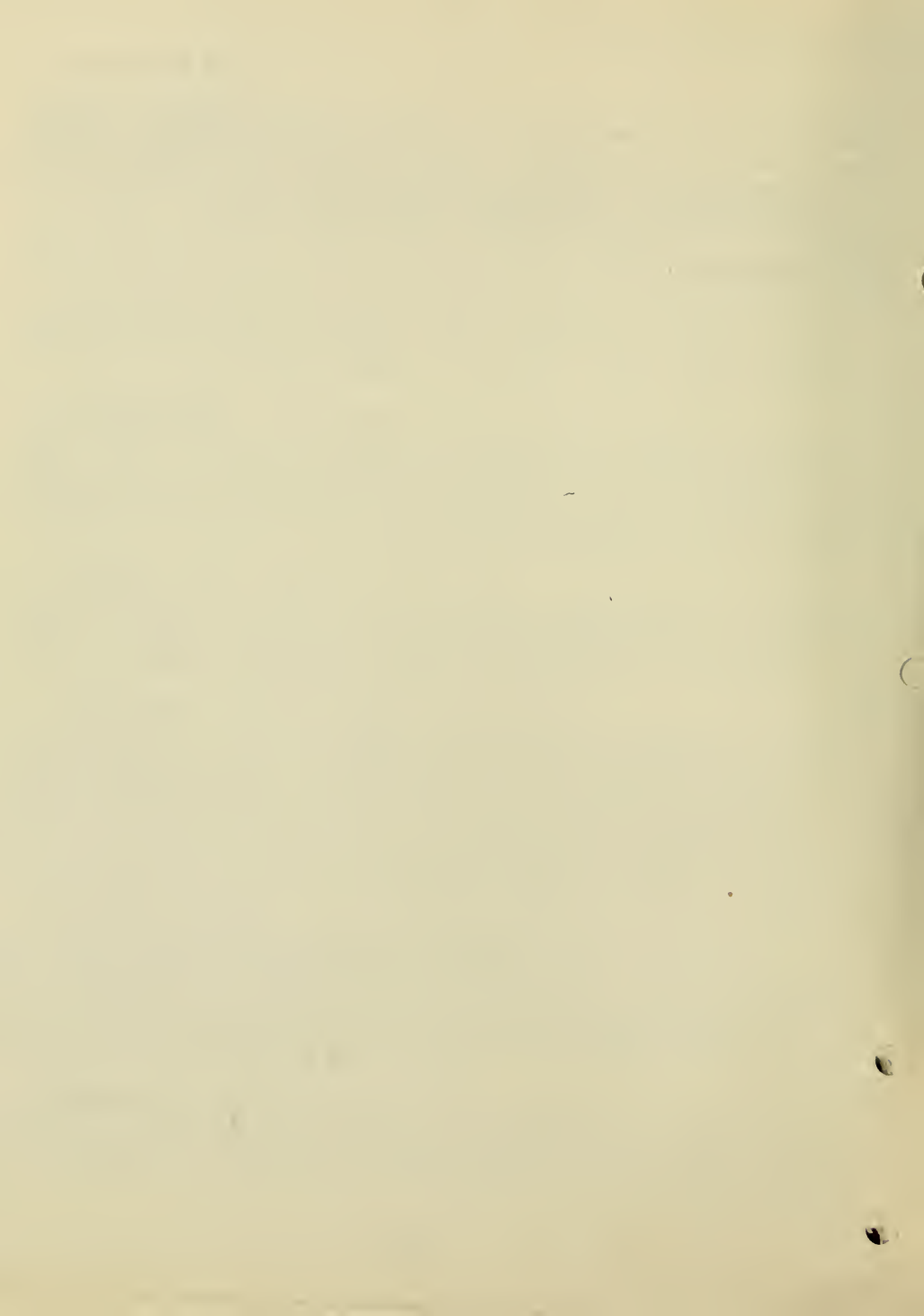
Major Findings: Several general trends emerged from the first full year of data collection. First, all hearing-impaired subjects showed poorer than normal temporal integration at frequencies where their audiometric loss was greater than 40 dB. Second, listeners with noise-induced hearing loss exhibited the greatest upward spread of masking in the continuous masker condition. Third, forward masking functions showed no major differences between groups of listeners. Finally, subjects with hearing loss tended to display an inability to follow the temporal contours of the masker in backward and gap masking conditions, suggesting poorer temporal resolution for these listeners.

Significance to NINCDS and Biomedical Research: These findings have obvious implications for the perception of speech by hearing-impaired listeners. The difference in masking patterns in the presence of noise suggest that these listeners would have more difficulty than normals understanding speech in noisy environments. These results also imply that improved versions of sensory aids will require more preprocessing of the temporal parameters of speech as well as intensity control.

Proposed Course: Data collection will be completed for the temporal masking conditions with signals and maskers in the second and third formant regions of speech. Studies examining temporal discrimination of narrow bands of noise will be conducted. In addition, the above-mentioned series of studies will be

performed at a lower intensity level in order to investigate temporal processing as function of this parameter. Speech discrimination in noise and in quiet will be measured for the purpose of relating our measures of temporal resolution to speech perception. It is expected that two manuscripts, one of which is near completion, will be published during the coming fiscal year.

PUBLICATIONS: None



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| SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space) | U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT | PROJECT NUMBER Z01 NS 02185-02 CDP (Prev. Proj. No.: Z01 NS 02185-01 C&ER) |
| PERIOD COVERED <div style="text-align: center;">July 1, 1975 to June 30, 1976</div> | | |
| TITLE OF PROJECT (80 characters or less) <div style="text-align: center;">Characteristics of Dysarthric Speech Associated with Neurologic Disease</div> | | |
| NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT | | |
| PI: C. L. Ludlow OTHER: V. Geoffrey R. Kartzinel D. B. Calne | Speech Pathologist Research Assistant Staff Fellow Clinical Director | CDP, NINCDS LP, NINCDS IR, NINCDS IR, NINCDS |
| COOPERATING UNITS (if any) | | |
| LAB/BRANCH <div style="text-align: center;">Communicative Disorders Program</div> | | |
| SECTION | | |
| INSTITUTE AND LOCATION <div style="text-align: center;">NINCDS, NIH, Bethesda, MD 20014</div> | | |
| TOTAL MANYEARS: <div style="text-align: center;">0.20</div> | PROFESSIONAL: <div style="text-align: center;">0.10</div> | OTHER: <div style="text-align: center;">0.10</div> |
| SUMMARY OF WORK (200 words or less - underline keywords) The long-range goal of this research is to develop objective measures for evaluating the type and severity of <u>speech production disorders</u> , different <u>dysarthrias</u> , associated with various neurological diseases. Measures made from <u>spectrographic analyses</u> of the <u>acoustic waveform</u> of dysarthric subjects' speech will be evaluated to determine which measures: | | |
| a) differentiate between the speech of Parkinson patients and normal adults of the same age and sex, b) differentiate between speech symptoms occurring in Parkinson patients in two different states; moderate to severe rigidity with bradykinesia and drug induced dyskinesia, c) differentiate between speech symptoms occurring in Parkinson patients receiving different drugs; <u>L-Dopa</u> and <u>Bromocriptine</u> , d) differentiate between the speech characteristics of patients with different neurological diseases; <u>Parkinson's disease</u> , <u>Huntington's chorea</u> , <u>amyotrophic lateral sclerosis</u> , and <u>cerebellar dysfunction</u> , e) correlate with perceptual judgments of the degree of speech production impairment, f) have adequate within subject test-retest reliability, and g) can be measured from spectrographic analyses of the same speech recording with minimal error. | | |

PROJECT DESCRIPTION:

Objectives: The goal of this project is to develop and evaluate measures of particular parameters of the acoustic waveform of speech which could be used for the assessment of speech production impairments associated with neurological disease.

Methods Employed: Identical testing conditions are maintained by presenting the task instructions and examples from a tape recording played at the same intensity setting on a tape recorder. Recordings are made while subjects perform tasks of extended phonation, loudness and pitch variation, pause and rate control, and rapid speech initiation. Measurements are made from the 34 sound spectrograms which result from analysis of each tape recording.

Subjects: Patients with Parkinson's disease were examined at two-week intervals over a six month period in a double-blind study of changes in medication. Presently, normal adult volunteers are being examined by the exact same procedures. In addition, a small number of patients with Huntington's Chorea and a few others with amyotrophic lateral sclerosis have been tested.

Major Findings: Measures which are made from sound spectrograms have been developed and are being evaluated for their validity and reliability in reflecting the type and degree of severity of different forms of dysarthria associated with neurological disease. These measures include:

- the duration of extended phonation,
- the change in rate of sentence production,
- the rate of phonation initiation and termination,
- the rate of syllable repetition,
- the change in rate of syllable repetition over 7 secs.,
- the latency of speech initiation,
- the average fundamental frequency of phonation during connected speech,
- the change in fundamental frequency during varying intonation,
- the change in syllable duration between stressed and nonstressed linguistic contrasts,
- the average sound pressure level of syllable production, and
- the range in sound pressure level of syllable production.

With the exception of the last two, all these measures have been made on at least two tape recordings of each of the 25 patients with Parkinson's disease included in the study. Data analyses are presently being conducted to determine which, if any, of the measures:

- a) differentiate between speech symptoms occurring in Parkinson patients in two different states (while receiving the same medication)-moderate to severe rigidity with bradykinesia and drug induced dyskinesia, and
- b) differentiate between speech symptoms occurring in Parkinson patients receiving different medications; L-Dopa and Bromocriptine.

Preliminary analyses indicate that two distinctive patterns of speech production can be identified with these measures; one associated with Parkinson patients when in a state of moderate to severe rigidity and the other with drug induced dyskinesia. Confirmation of these initial findings awaits completion of the normal data. Then it can be determined which measures indicate particular deficits in speech production occurring during each of the two states, rigidity and dyskinesia.

Significance to NINCDS and Biomedical Research: The development of objective procedures for the assessment and differential diagnosis of various types of dysarthria in adults with neurological diseases, will enable researchers and clinicians to evaluate different treatments for these patients. Information is being gained also on which aspects of the speech production process are particularly impaired in each of the neurological diseases under study. The results will contribute to our understanding of the processes of both normal and disordered speech production as well as providing directions for treatment.

Proposed Course of Project: Subject testing of normal adults will be completed by July, 1976. Data processing and analysis is expected to continue until September, 1976. Papers will be submitted for journal publication in the early fall of 1976.

Similar methods of study will be used to determine the effects of dipropylacetate (Depakine) on the speech production impairments of patients with cerebellar deficits.

Publications: None

ANNUAL REPORT
July 1, 1975 through June 30, 1976
Fundamental Neurosciences Program
National Institute of Neurological and
Communicative Disorders and Stroke

With the reorganization of the Extramural Programs of the NINCDS into a number of disease categories, individual grants and contracts have been coded to identify their relationship to particular groups of disorders of the nervous system and so have been divided into different programs. Those grants and contracts which have longer range relationships to specific disorders and tend to emphasize the exploration of the normal nervous system rather than any particular pathology have been coded as fundamental neuroscience studies and make up the Fundamental Neurosciences Program.

Staff

There are currently four neuroscientists and three secretaries on the FNP staff and an additional scientist is being recruited. Each of these scientists is professionally trained in one or more of the neural specialties: Neurophysiology, Biophysics, Bioengineering, Medicine, Neuropathology and Psychology. This group manages a program consisting of some 378 research grants at a cost of about \$23 Million and 14 contracts for about \$1.5 Million.

Even within the FNP there is wide variability in the lead time required before research findings can be applied to treatment of disease. For example, there is increased effort by grantees in application of computers to neurobiological problems, problems of pattern recognition in studying how groups of nerve cells work together to control muscle movement, problems of computer assisted analysis of brain waves (EEG). Sleep is classified into different types and the normal and abnormal balance between these different types is studied with the help of computers.

Basic mechanisms in sensory neurophysiology must be understood in order to learn how to preprocess sound so that electrical stimulation of the nervous system of deaf individuals can be utilized for intelligible speech communication.

Some of the most fundamental studies in FNP relate to the manner in which interconnections between nerve cells affect the function of the nervous system. The nervous system is not "hard-wired" like a telephone switchboard, rather, it is thought, the synapses or connections at nerve endings continue to change with time, new ones forming and old ones dissolving. Such changes in "connectivity" are stimulated by changes in usage or activity of nerve pathways. Increased efforts are planned for these studies of the functional modification of the nervous system with usage.

RESEARCH GRANTS

The total range of FNP grant supported research is too broad to summarize effectively. Instead, noteworthy examples are referred to in order to illustrate important research results reported by grantees:

Neurophysiology and Biophysics

Neurophysiologists concerned with the role of nerve cells of the brain in control of movement have discovered a number of new facts that make clearer the basic mechanisms involved. Because of damage caused by microelectrodes used to record the activity of nerve cells there is a systematic sampling bias which has misled researchers into overemphasizing the role of large pyramidal tract nerve cells in motor control. Using an appropriate sampling bias correction formula now shows that a different group of smaller, slower conducting cells and fibers is primarily concerned with cortical control of movement.

When the activity of large numbers of single cells is recorded simultaneously, it is possible to study the correlation of spike activity in different cells. Such studies permit insight into the basic mechanisms by which nerve cells in the same or different parts of the brain interact and coordinate their activities. Through specially developed mathematics these correlation techniques are being used to study the "preset state" of the nervous system before execution of a particular task. The resulting patterns of nerve cell activity reveal the dynamic behavior of neuronal functional groupings and so may lead to further insight into how the brain controls movement.

Other researchers have shown that the varying interactions of groups of muscle fibers called motor units make the mechanical responses to repeated patterns of nerve signals variable and dependent on the state of the muscle. Consequently, the hypothetical control scheme used by the CNS to determine motor activity must not operate by combining "known" individual motor unit responses as has been previously proposed.

At a more fundamental level the biophysics of the excitable nerve cell membrane has been further explored. During the generation of an action potential or nerve spike it has long been known that the membrane becomes permeable to ions such as sodium or potassium. New work supported by NINCDS has revealed that these excitable channels probably exist in only two states, open or closed, rather than in a number of states in which their electrical conductance varies with the electrical potential across the membrane. Such esoteric details of neurobiology may seem far removed from the immediate problems of diseases of the nervous system, but they are essential to a thorough understanding of how the normal nervous system works.

Isolation of the Acetylcholine Receptor

One of the most significant advances in neurochemistry has been the isolation, purification and characterization of the acetylcholine receptor protein from the post-synaptic membrane. While the favorite starting material is the electroplax of the electric ray (Torpedo), receptor protein has also been obtained from muscle fibers and rat brain. A number of glycoproteins of varying molecular weight have been obtained. It is not as yet known whether these are similarly constituted polymers, perhaps artificially produced by the isolation procedure or if the native receptor consists of a number of different specific polypeptide chains linked together. Studies currently under way include the kinetics and ligand binding and associated conformational changes in structure, the distribution of receptor density along the muscle membrane with respect to innervation, the molecular mechanisms of action of cholinergic drugs and toxicants and the link between receptor activity and the opening of the sodium channel.

A number of studies are particularly noteworthy. Several laboratories have demonstrated that the injection of acetylcholine receptor protein into animals produces a myasthenia-like syndrome, apparently the result of antibody binding to the receptors of muscle. These reports, confirmed independently by others, form the basis of current theories on the etiology of myasthenia gravis. Neurotoxins which bind to the acetylcholine receptor have been conjugated with horseradish peroxidase, applied to toad muscle and brain, and shown with electron microscopy to bind to the sub-synaptic membrane, the receptor site. Finally, purified receptor has been incorporated into artificial phospholipid membranes which demonstrate an enhanced flux of cations such as Na^+ upon interaction with carbamylcholine but is blocked in this respect by d-tubocurarine.

Retrograde Axoplasmic Transport

The apparent movement of material from the nerve cell body distally toward the axon terminals had been classically described and analyzed more than twenty-five years ago by Weiss. In recent years it has been established that various components flow at different speeds, the metabolic substrate supporting the process has been partially elucidated, and that the mechanism can be inhibited by a variety of agents. Current research along these lines has been oriented toward identifying the proteins, particles and organelles which are distally transported.

Relatively recently, it was noted that axon transport also occurs in the reverse (retrograde) direction and may be the mechanism whereby viruses and other substances (i.e., tetanus toxin) spread from the peripheral into the central nervous system. The capacity of axon terminals to take up horseradish peroxidase forms the basis for tracing neuronal pathways through the central nervous system.

Currently, anterograde and retrograde transport are under study relative to each other. Preliminary observations suggest that particles moving retrograde are larger, more electron dense and differently shaped, supporting the logical idea that different substances and structures are transported in different directions. The nature of the transport channels is also under investigation--they may consist of groups of microtubules or perhaps tubules of endoplasmic reticulum. The process of endocytosis at presynaptic terminals is stimulated by the presence of acetylcholine or strychnine and retrograde transport is diminished by podophyllotoxin, a drug known to interact with neurotubules. One interesting question under study is whether endocytotically retrieved synaptic vesicles may be reused for neurotransmission.

Contractile Proteins in the CNS

Several years ago, it was noted that certain protein fractions derived from brain possessed contractile properties analogous to those observed in vitro with muscle proteins. More recently a number of new, purified moieties have been obtained from nervous tissue belonging to the family of contractile and relaxing proteins. Biochemical, biophysical and immunological characterization of these substances is currently proceeding along orthodox lines of investigation primarily by comparison with contractile proteins isolated from other tissues. Antibodies to these entities have been produced and their subcellular localization by both light and electron microscopy is currently in progress. However, it has already been established that a significant concentration of these proteins is localized in synaptosomes, suggesting that they may be functionally related to the release of neurotransmitters from axon terminals. If so, the contractile mechanisms at nerve endings may represent the culmination of the excitation and conduction processes in nerve.

Evolution of the Nervous System

Grant supported research is under way to uncover the evolutionary modifications that have occurred to produce the so-called sensory cortex of primates. One set of recent experiments has led to the conclusion that the auditory cortex of the monkey plays little if any role in the strictly sensory aspects of hearing, rather the impairment after removal of the cortical tissue is that the animal fails to maintain complex or involved responses to auditory signals.

Brain Stimulation

Electrical stimulation of the brain can affect appetite and other motivational aspects of rat behavior. It is possible to distinguish between the motivational property of electrical stimulation of the lateral hypothalamus and the ordinary appeal of food by pairing the different conditions with lithium chloride injections (which make the animals sick). Animals receiving

brain stimulation do not show the usual aversion found 48 hours after the injection. Adjustment to the optimal electrical pulse rate has demonstrated that the animal can detect signals weak enough to be considered appropriate for prosthetic devices to be attached to the central nervous system.

Habituation

Biochemical investigations have been supported on the nature of habituation discovered in the protozoan Stentor. It has been shown that the curariform drugs selectively block the animal's sensitivity to mechanical stimulation. By comparing habituated animals with other untrained animals, it was possible to demonstrate the probable site of action of different drugs and thereby localize the site of the change that occurs with this form of adaptation or habituation.

RESEARCH CONTRACTS

Neural Prosthesis Program

There is no doubt that the steady growth of techniques and devices (neural prostheses) for relieving the neural deficits of the handicapped patient is the way of the future.

Ten contracts have been let to study the most fundamental problems which are common to all neural prostheses.

The effects of long-term electrical stimulation of the central nervous system in animals are being studied with various electrode designs, stimulus waveforms and stimulus parameters. These studies include the effects on the blood brain barrier and regional cerebral blood flow. Following stimulation, the meninges, nervous tissue and associated vasculature are examined histopathologically with both the light and electron microscope.

Single unit recording from visual cortical units during stimulation is utilized to determine the mechanisms and extent of cellular excitation in cats. When human cases become available which require occipital craniotomies, the visual cortex is stimulated to produce phosphenes. In this way the information on the effects of various stimulation parameters and electrode configurations is obtained for design of cortical neural prostheses.

The stability of the excitability of the visual cortex during long-term electrical stimulation is being studied in monkeys. The mechanisms causing increases in threshold for detection of stimulation and means of preventing the increases are being evaluated. The effects of chronic blindness and various stimulus modulation schemes on information transfer rates are also being studied.

New electrode materials are being evaluated by both in vitro and in vivo testing in animals. Following the in vivo testing, the brain tissue is subjected to histopathological examination.

Both animal studies and muscle implant studies in humans are directed toward the development of proportional control of the upper extremities in paralyzed individuals. In particular, methods of reversing disuse atrophy, preventing muscle fatigue and providing smooth, coordinated muscle contractions are being investigated. The possibility of using stimulation of the paraspinal muscles to correct scoliosis is undergoing initial trials in animals.

The electrochemical properties of both metal and capacitor stimulating electrodes are being studied. In particular, potential toxic reaction products and their relation to electrode design and stimulus parameters are being evaluated.

Using alumina cream implants in the hippocampus of monkeys, a model of temporal lobe epilepsy has been developed. The effects of cerebellar stimulation on the behavioral aspects of these seizures and on the single unit firing behavior of hippocampal single units is being studied. Also the neurophysiological mechanisms and anatomical pathways associated with the cerebellar stimulation are being determined.

Implanted electronic devices can be used to stimulate the spinal cord and its nerve roots in such a way that urinary bladder evacuation occurs. Failure of the bladder to drain properly is one of the major causes of urinary infection and death in paraplegic patients. Thus, in this area of fundamental studies of the normal bladder reflex mechanism and the development of successful techniques for nervous system stimulation by remote control may lead very directly to relief of human disease.

Biomedical Engineering

The possibility of using ultrasonic techniques for the detection and characterization of intracranial lesions is being investigated. Emphasis has been placed on developing a method of determining an impedance profile of tissue and on characterizing the effects of intervening skull bone on the ultrasonic signals. Emphasis has been placed on determining the acoustic properties of normal and pathological tissue and on developing the moving target indicator technique for assessing cerebral blood flow in the major cerebral arteries.

Multichannel transdermal stimulators are being developed for auditory prostheses.

Neural Prosthesis Workshop

A workshop on the application of functional electrical stimulation to neural prostheses was held at Kellogg West Conference Center from May 17-19, 1976 in Pomona, California. It was jointly sponsored by the Rehabilitation

Services Administration and our Neural Prosthesis Program, NINCDS.

Participants included investigators from the United States and Europe who are working on either basic studies related to neural prostheses or their actual clinical development.

Attention focused on skeletal muscle activation for the paralyzed, detrusor activation for patients with neurogenic bladders, cerebellar stimulation for epilepsy and movement disorders, visual prostheses for the blind and auditory prostheses for the deaf. The papers and discussion is being edited by our staff and published as a book.

CONTRACT NARRATIVE
Fundamental Neurosciences Program, EP, NINCDS
July 1, 1975 - June 30, 1976

UNIVERSITY OF FLORIDA (N01-NS-1-2286)

Title: Electrode Materials Study

Contractor's Project Director: William W. Dawson, Ph.D.

Current Annual Level of Support: \$69,148

Objectives: These investigators are evaluating presently available materials for the construction of electrodes both in vitro and in vivo as well as developing new electrode materials.

Major Findings: Antimony-doped stannous oxide is extremely resistant to corrosion in vitro and may make a good electrode material. Histopathological examination of neural tissue after stimulation through capacitor electrodes has revealed little or no damage when using stimulus pulses with relatively low charge per phase.

Significance to Biomedical Research and to the Program of the Institute:
The evaluation and development of electrode materials is necessary for all devices that utilize electrical stimulation of excitable tissue.

Proposed Course of Contract: The histopathological evaluation of capacitor electrodes utilizing relatively high charge levels per phase is planned. Also the development of new, flexible, biocompatible, non-leaky insulators is required.

CONTRACT NARRATIVE
Fundamental Neurosciences Program, EP, NINCDS
July 1, 1975 - June 30, 1976

UNIVERSITY OF CALIFORNIA AT SAN FRANCISCO (N01-NS-3-2307)

Title: Studies of Urinary Bladder Evacuation by Electrical Stimulation

Contractor's Project Director: Emil Tanagho, M.D.

Current Annual Level of Funding: \$38,020

Objectives: Studies are being conducted in animals with upper motor neuron lesions to determine the feasibility of urinary bladder evacuation by electrical stimulation of the sacral roots.

Major Findings: Effective contraction of the detrusor muscle has been obtained by stimulation of the sacral roots. However, the problem of simultaneous contraction of the striated sphincter has not been completely overcome.

Significance to Biomedical Research and the Program of the Institute: The ability of a person with a neurogenic bladder to empty the bladder voluntarily is the long-range goal of this work.

Proposed Course of Contract: Methods of overcoming sphincter contraction during bladder evacuation will be pursued.

CONTRACT NARRATIVE
Fundamental Neurosciences Program, EP, NINCDS
July 1, 1975 - June 30, 1976

UNIVERSITY OF CALIFORNIA LOS ANGELES (N01-NS-4-2331)

Title: Studies on the Effects of Electrical Stimulation of the Cerebellum

Contractor's Project Director: Thomas Babb, Ph.D.

Current Annual Level of Funding: \$112,200

Objectives: The effects of cerebellar stimulation on the behavioral aspects of seizures produced by alumina cream implants in the hippocampus of monkeys and on the single unit firing behavior of hippocampal single units are being studied.

Major Findings: A model of psychomotor epilepsy in the monkey has been demonstrated. Of eight monkeys that developed seizures, two have received cerebellar stimulation. The animals continued to have spontaneous psychomotor seizures although there is some evidence to suggest that their intensity and duration may be reduced. No evidence of rebound has been found following cessation of stimulation. In the cat, neither membrane responses of neurons in the hippocampus nor field potentials of fibers and neurons in the region of the hippocampus have exhibited any changes during or following electrical stimulation of the cerebellar surface or deep nuclei.

Significance to Biomedical Research and to the Program of the Institute: These studies should provide information on the mechanisms, if any, by which cerebellar stimulation modifies clinical seizures and movement disorders.

Proposed Course of Contract: Studies will be continued in monkeys who have received hippocampal implants. Cerebellar stimulation will be evaluated in terms of the effects on both the electrical and behavioral manifestations of the seizures. Evaluation of a possible direct cerebellar-hippocampal pathway will be made on a single unit level.

CONTRACT NARRATIVE
Fundamental Neurosciences Program, EP, NINCDS
July 1, 1975 - June 30, 1976

STANFORD UNIVERSITY (N01-NS-5-2306)

Title: Transdermal Stimulation Electronics for an Auditory Prosthesis

Contractor's Project Director: Robert White, Ph.D.

Current Annual Level of Support: \$89,000

Objectives: The design and development of transdermal stimulators to be used in the evaluation of multi-channel cochlear implant auditory prostheses.

Major Findings: Prototype four-channel transdermal stimulators have been constructed and are undergoing in vitro and in vivo testing.

Significance to Biomedical Research in Programs of the Institute:
The Institute is presently supporting under the grant mechanism the evaluation of multi-channel auditory prostheses. This contract will provide electronic stimulators to these grantees.

Proposed Course of Contract: A second generation multi-channel stimulator will be designed and developed on the basis of specifications derived from experience with the present system.

CONTRACT NARRATIVE
Fundamental Neurosciences Program, EP, NINCDS
July 1, 1975 - June 30, 1976

UNIVERSITY OF MINNESOTA (N01-NS-4-2332)

Title: Study of the Effects of Electrical Stimulation of the Cerebellum

Contractor's Project Director: Heinrich Bantli, Ph.D.

Current Annual Level of Support: \$86,063

Objectives: The effects of cerebellar stimulation on primate models of epilepsy are being evaluated. Also the neurophysiological mechanisms and anatomical pathways associated with the cerebellar stimulation are being determined.

Major Findings: Stimulation of the cerebellar surface in cats activates afferent fibers that synapse with Purkinje cells and through collaterals synapse on the neurons of the deep cerebellar nuclei. Such stimulation also can activate neurons in the ascending reticular formation. A projection from the dentate nucleus to the medial reticular formation has been confirmed. A projection from the cerebellar deep nuclei to the cerebellar cortex was discovered. To date, cerebellar stimulation has had no directly observable or statistically detectable effect on the firing patterns of single units in the vicinity of epileptic foci in monkeys.

Significance to Biomedical Research and the Program of the Institute: These studies should provide information on the neurophysiological mechanisms, if any, by which cerebellar stimulation modifies clinical seizures and movement disorders.

Proposed Course of Contract: The work described will continue to be pursued as well as an evaluation of the neurological function of patients who have received cerebellar implants under a separate project not supported by this contract.

CONTRACT NARRATIVE
Fundamental Neurosciences Program, EP, NINCDS
July 1, 1975 - June 30, 1976

MASSACHUSETTS GENERAL HOSPITAL (N01-NS-0-2276)

Title: Studies to Determine the Feasibility of a Sensory Prosthesis

Contractor's Project Director: Daniel Pollen, M.D.

Current Level of Funding: \$40,850

Objectives: The mechanisms of neuronal activation resulting from electrical stimulation of the central nervous system are being studied. Methods of reducing the latency of activation of neurons and methods of preventing after discharges are being developed. Also the subjective experiences of human patients during stimulation of the visual cortex are being studied.

Major Findings: Intracellular recording from several intracortical neurons during surface stimulation has been accomplished. There is some evidence that these cells were directly activated rather than synaptically activated.

Significance to Biomedical Research and to the Program of the Institute: An understanding of the mechanisms of activation of neurons by electrical stimulation and the subjective experiences of such activation are important for the development of neural prostheses for the neurologically handicapped.

Proposed Course of Contract: Much work remains utilizing the technique of intracellular recording during stimulation. Also as human cases become available more information will be obtained on methods of directly communicating with the central nervous system by means of electrical stimulation.

CONTRACT NARRATIVE
Fundamental Neurosciences Program, EP, NINCDS
July 1, 1975 - June 30, 1976

HUNTINGTON INSTITUTE OF APPLIED MEDICAL RESEARCH (N01-NS-0-2275)

Title: Studies to Determine the Feasibility of a Sensory Prosthesis

Contractor's Project Director: Robert Pudenz, M.D.

Current Annual Level of Funding: \$123,000

Objectives: The effects of long-term electrical stimulation of the nervous system in animals are being studied with various electrode designs, stimulus wave forms, and stimulus parameters. These studies include the effects on the blood brain barrier and regional cerebral blood flow. Following stimulation, meninges, nervous tissue and associated vasculature are examined histopathologically with both the light and electron microscope.

Major Findings: Rhodium metal implanted passively on the cerebral cortex has not produced the tissue damage reported by other investigators. Tissue damage has been caused by platinum salts indicating that corrosion products from platinum electrodes may be toxic.

Significance to Biomedical Research and to the Program of the Institute: These studies are important for determining the safety and efficacy of various forms of stimulation of the central nervous system for use in neural prostheses for the neurologically handicapped.

Proposed Course of Contract: Work is required both in understanding the mechanisms of neural damage as well as means of safely stimulating the nervous system.

CONTRACT NARRATIVE
Fundamental Neurosciences Program, EP, NINCDS
July 1, 1975 - June 30, 1976

CASE WESTERN RESERVE UNIVERSITY (N01-NS-2-2314)

Title: Study of Intramuscular Electrical Stimulation of Muscle

Contractor's Project Director: Thomas Mortimer, Ph.D.

Current Annual Level of Funding: \$208,000

Objectives: Both animal studies and muscle implant studies in humans are directed toward the development of proportional control of the upper extremities in paralyzed individuals. In particular, methods of reversing disuse atrophy, preventing muscle fatigue and providing smooth, coordinated muscle contractions are being investigated. The possibility of using stimulation of the paravertebral spinal muscles to correct scoliosis is undergoing initial trials in animals.

Major Findings: A method of producing fine motor control by combining first pulse width modulation at low force levels followed by pulse frequency modulation at high force levels has been demonstrated. The determination of safe charge limits for in vivo muscle stimulation with stainless steel electrodes has been made.

Significance to Biomedical Research and to the Program of the Institute: The techniques being investigated are intended to restore lost function in paralyzed individuals and lead to an effective treatment for scoliosis.

Proposed Course of Contract: A totally implanted stimulator for finger flexion and extension in humans is being developed. The provision of feedback information other than visual feedback is a natural extension and will be investigated.

CONTRACT NARRATIVE
Fundamental Neurosciences Program, EP, NINCDS
July 1, 1975 - June 30, 1976

EIC CORPORATION (N01-NS-3-2313)

Title: Safe Procedures for Electrical Stimulation of the Nervous System

Contractor's Project Director: Barry Brummer, Ph.D.

Current Annual Level of Funding: \$85,574

Objectives: The electrochemical properties of both metal and capacitor electrodes are being studied. Potential toxic reaction products and their relationships to electrode design and stimulus parameters are being evaluated.

Major Findings: Tests in inorganic saline solution have shown that neither chloride oxidation nor pH shifts are likely to constitute significant problems during biphasic stimulation with platinum electrodes. However, metal dissolution is a more difficult problem to avoid. Platinum dissolution has been monitored by ultraviolet spectro-photometric analysis and related to the charge density per pulse and the current density.

Significance to Biomedical Research and the Program of the Institute: The development and evaluation of safe stimulating techniques for use in neural prostheses is one of the major goals of the Neural Prosthesis Program of the Institute.

Proposed Course of Contract: Development of intracortical electrodes and the electrochemical evaluation of stainless steel electrodes for intramuscular electrodes will be carried out during the coming year.

CONTRACT NARRATIVE
Fundamental Neurosciences Program, EP, NINCDS
July 1, 1975 - June 30, 1976

UNIVERSITY OF ROCHESTER (N01-NS-0-2279)

Title: Development of a Sensory Prosthesis

Contractor's Project Director: Robert Doty, Ph.D.

Current Annual Level of Funding: \$81,587

Objectives: The stability of the excitability of the visual cortex during long term electrical stimulation is being studied in monkeys. The mechanisms causing increases in threshold for detection of stimulation and means of preventing the increase are being evaluated. The effects of chronic blindness and various stimulus modulation schemes on information transfer rates are also being studied.

Major Findings: All forms of electrical stimulation of the striate cortex, even those below the threshold for detection of stimulation, produce an increase in the threshold for detection if the stimulation is applied continuously for 0.5 to 1 hour. This deleterious effect of protracted stimulation spreads laterally for several millimeters from the stimulated site, raising the threshold at non-stimulated loci.

Significance to Biomedical Research and to the Program of the Institute: This work will be useful for developing safe and efficient methods of stimulating the central nervous system for use in neural prostheses for the neurologically handicapped.

Proposed Course of Contract: The investigators will attempt to find techniques of electrical stimulation which will be effective but non-injurious even when applied continuously for long periods of time. They will also investigate whether the "kindling" phenomenon results from electrolytic effects or whether it is a true reorganization of neural activity as claimed by other investigators.

CONTRACT NARRATIVE
Fundamental Neurosciences Program, EP, NINCDS
July 1, 1975 - June 30, 1976

UNIVERSITY OF CALIFORNIA AT LOS ANGELES, BRAIN INFORMATION SERVICE
(UCLA - BIS) (N01-NS-3-2306)

Title: Operation of Specialized Information Center in Brain
and Other Neurosciences

Contractor's Project Director: Michael H. Chase, Ph.D.

Current Annual Level of Support: \$360,000

Objectives: The Contractor operates a specialized information center which serves as a national focal point for the identification, collection, storage, retrieval, analysis, repackaging, and dissemination of information on non-clinical neurosciences. The major thrusts of this information center are information analysis products and services using the identified and stored information. The Contractor makes available comprehensive information services, including: a) current alerting bulletins, b) demand searches of the data base, and c) other products and services mutually agreed upon by the Contractor and the Institute.

Major Accomplishments: During the current contract period, the Brain Information Service is carrying out the following activities:

Current Alerting Bulletins

Biogenic Amines and Transmitters
Neuroendocrine Control Mechanisms
Sleep Bulletin and Sleep Review
Developmental Neurobiology (first issue, March 1976)

Recurrent Bibliographies

Cerebral Evoked Potentials
Proteins in the Brain
Inborn Errors of Metabolism: Physiology and Biochemistry
Neuroimmunology

Reference Bibliographies

Conference Reports

Winter Conference on Brain Research, 1976
Society for Neuroscience Annual Meeting, 1975
Conference on Human Brain Function

Annual Bibliographic Cumulations

Biogenic Amines in the Central Nervous System, 1975
Hypothalamic-Pituitary-Gonadal System, 1975
Electrical Recordings in the CNS and Related Literature
(quarterly and cumulative), 1975

Memory and Learning Neural Correlates in Animals, 1975
Sleep Research, Volume 5, 1976

Updated Reviews

Neuronal Activity in Sleep

Special Reports

Enzyme Therapy in Organic Lesions of the Spinal Cord
L.A. Matinian and A.S. Andressian (translation)

Research Reports

Cortical Organization
Paul Crandall and Thomas Babb

Soviet Research Reports

Cortical Plasticity by L.L. Voronin
Anatomy of the Auditory System by T.A. Mering

Significance to Biomedical Research and the Program of the Institute: The Brain Information Service performs an important service to the biomedical community by the maintenance of a very extensive data base devoted to the fundamental neuroscience literature. In addition, a number of important synthetic and analytic information products are produced by the staff for distribution to the neuroscience community.

Proposed Course of the Contract: The program is under the continuing surveillance of the NINCDS Project Officer. In addition, a BIS National Scientific Advisory Committee has been formed and has met twice. Each product receives continuing detailed review.

CONTRACT NARRATIVE
Fundamental Neurosciences Program, EP, NINCDS
July 1, 1975 - June 30, 1976

BOWMAN GRAY SCHOOL OF MEDICINE (N01-NS-4-2304)

Title: Study and Test of Ultrasound Techniques for
Diagnosis of Cerebral Disorders

Contractor's Project Director: Ralph W. Barnes, Ph.D.

Current Annual Level of Support: \$84,751

Objectives: One of the major problems in effectively studying intracranial arterial echo activity, either in range or amplitude, has been isolating the arterial echo and measuring its activity during the cardiac cycle. The isolation of intracranial artery echoes and their activity has been achieved using moving target indicator (MTI) techniques originally developed for radar and sonar use. The Contractor is extending the basic MTI technique in order to develop and clinically evaluate instrumentation for non-invasive assessment of cerebrovascular dynamics.

Major Findings: At present, intracranial echoes can be detected from the internal carotid arteries at the circle of Willis, the middle cerebral arteries, the anterior cerebral arteries, and the vertebral arteries. Preliminary determinations have been made of the delay time of the arrival of the echo at the common carotid and at the internal carotid relative to the R-wave of the ECG in subjects with no known vascular disease and from one patient with a known transient ischemic attack.

Significance to Biomedical Research and the Program of the Institute: The diagnosis and treatment of stroke and other intracranial disorders is a major concern to NINCDS and to the medical fields related to the Institute's mission. The non-invasive diagnostic techniques being investigated under this project will, if successful, provide valuable tools for non-invasive diagnosis of major forms of cerebrovascular disease.

Proposed Course of Contract: Future work on this project will be in two areas: 1) Further study of intracranial artery echo activity using time motion and moving target indicator techniques. Differences between patients with no known vascular disease and patients with known vascular disease or other intracranial pathology will be studied. 2) Development and use of scanning techniques using MTI processing to image the intracranial arteries.

CONTRACT NARRATIVE
Fundamental Neurosciences Program, EP, NINCDS
July 1, 1975 - June 30, 1976

INDIANAPOLIS CENTER FOR ADVANCED RESEARCH (ICFAR) (N01-NS-3-2319)

Title: Study and Test Ultrasonic Techniques for
Diagnosis of Cerebral Disorders

Contractor's Project Director: Francis J. Fry

Current Annual Level of Funding: \$85,294

Objectives: This project is being performed as a joint venture by the Indianapolis Center for Advanced Research (ICFAR) and Bolt, Beranek, and Newman (BBN). The scope of work includes: 1) Making measurements of acoustic transfer functions of human skull utilizing various skull regions and angles of incidence. The following properties are being measured: reflectivity, transmission loss, phase speed, group speed, and scattering. 2) Making acoustic measurements on solid ivory skull pieces and on prepared volumes of diploe in which the solid ivory overlays have been removed. 3) Making a series of measurements on physical models of bone to aid in identifying the mechanisms which cause the observed skull acoustical properties.

Major Findings: Preliminary measurements of a number of acoustic parameters have been made on pieces of fresh and formalin-fixed human skull bone. Insertion loss, transmission phase shift, and reflection loss have been measured as a function of frequency. The degree of spreading of the sound beam caused by the skull has been determined. Based on these measurements and on the physical parameters of human skull bone, Dr. James Barger (BBN) has developed a multiple transmission line model of human skull and a scattering model of beam spreading. Work so far suggests that measurement of scattering properties of intracranial contents at frequencies below 1MHz may be a useful first step toward visualization of intracranial contents in the adult.

Significance of Biomedical Research and to the Program of the Institute: The diagnosis and treatment of stroke, head injury, brain tumor, and other intracranial disorders is of prime interest to NINCDS and the scientific fields that it represents. It is felt that non-invasive techniques, both for screening for cerebral disorders and for diagnosis of specific pathological lesions and their serial assessment following treatment, would be of great use in improving the clinician's capabilities in diagnosis and treatment of these disorders. If successful, the techniques being investigated under this contract will provide an extended capability for non-invasive assessment of brain tissue status.

Proposed Course of Contract: The next phase of the work will involve more detailed physical measurements of acoustical parameters and refinement of the analytic model of human skull.



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